Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 39, No. 12, December 1982

Volume 39, n° 12, décembre 1982

Canada

Fisheries and Oceans / Pêches et Océans

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

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Direction de l'information et des publications scientifiques
Ottawa K1A 0E6

Volume 39, No. 12, December 1982

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Canadian Journal of Fisheries and Aquatic Sciences

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Announcements / Avis

Index

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Index

Un index par sujet et auteur et une liste de toutes les publications parues en 1982 paraîtront au début de 1983 et seront distribués gratuitement à tous ceux qui reçoivent le *Journal*, ainsi qu'aux abonnés. On pourra se procurer des exemplaires supplémentaires à la Direction de l'information et des publications scientifiques.

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Effect of Temperature on Initial Feeding in Alevins of Chinook Salmon (*Oncorhynchus tshawytscha*)

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HEMING, T. A., J. E. MCINERNEY, AND D. F. ALDERDICE. 1982. Effect of temperature on initial feeding in alevins of chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 39: 1554–1562.

Growth and survival of chinook salmon (*Oncorhynchus tshawytscha*) during the transition to active feeding were examined in relation to the timing of initial food presentation at 6, 8, 10, and 12°C. The transition to active feeding in these salmon involved a period of "precocious feeding" during which ingestion of food had no effect on fish size, growth rate, or survival. Initial utilization of food for growth and survival did not coincide with any specific stage of development, but rather it occurred earlier and at a less mature stage of development at higher temperatures. Delays in initial food presentation beyond this point reduced fish size and survival. Temperature and the timing of initial food presentation interacted to create a zone of optimum feeding. Biomass production was maximized when alevins began to feed within this zone, independent of the precise time of initial food presentation or temperature. This optimum feeding zone existed at temperatures below 12°C, between 905 thermal units (tu) postfertilization and a point (F, tu) which varied with temperature (T, °C) as $F = 1201.1 - 20.3\ T$.

Key words: temperature, initial feeding, *Oncorhynchus tshawytscha*, fish culture

HEMING, T. A., J. E. MCINERNEY, AND D. F. ALDERDICE. 1982. Effect of temperature on initial feeding in alevins of chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 39: 1554–1562.

La croissance et la survie de saumons chinook (*Oncorhynchus tshawytscha*) pendant la période de transition vers une alimentation active ont été étudiées en fonction du moment de la présentation initiale de nourriture à 6, 8, 10 et 12°C. La transition comporte une période d'« alimentation précoce », au cours de laquelle l'ingestion de nourriture n'influe pas sur la taille, le taux de croissance ou la survie. L'utilisation initiale de la nourriture pour la croissance et la survie ne coïncide avec aucun stade spécifique de développement, mais survient plus tôt et à un stade de développement moins avancé aux températures plus élevées. Si la présentation initiale de nourriture est retardée au-delà de ce point, la taille et la survie des poissons sont diminuées. La température et le moment de la présentation initiale de nourriture agissent de concert pour créer une zone d'alimentation optimale. La production de biomasse est portée au maximum quand les alevins commencent à se nourrir dans les limites de cette zone, indépendamment du moment précis de la présentation initiale de nourriture ou de la température. Cette zone existe à des températures inférieures à 12°C, entre 905 unités thermiques (tu) après fécondation et un point (F, tu) variant en fonction de la température (T, °C) selon $F = 1\ 201{,}1 - 20{,}3\ T$.

Received December 9, 1981
Accepted July 27, 1982

Reçu le 9 décembre 1981
Accepté le 27 juillet 1982

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Printed in Canada (J6750)
Imprimé au Canada (J6750)

AVAILABILITY of adequate external food at the time when yolk absorption becomes nutritionally limiting is a major determinant of early growth and survival of fishes (May 1974). Even short periods of food deprivation at that time can result in abnormal behavioral and morphological development, degen-

eration of the alimentary tract and trunk musculature, and reductions in food utilization efficiency and feeding activity (Blaxter and Hempel 1963; Toetz 1966; Blaxter and Ehrlich 1974; Theilacker 1978). Consequently, the question of when active feeding normally begins is important, especially in fish culture where food availability can be manipulated.

In salmonids, the precise timing of initial feeding is uncertain. Previous studies have related the onset of feeding in salmon and trout to a wide variety of events: emergence from the gravel redd and initial inflation of the gas bladder (Palmer et al. 1951; Stuart 1953; Dill 1967; Bams 1969; Twongo and MacCrimmon 1976); attainment of maximum alevin wet weight (Marr 1965); disappearance of the visible yolk sac or "button-up" (Henshall 1904; Atkins 1905, 1906; White 1915; Brown and Buck 1939; MacCrimmon and Twongo 1980); complete yolk absorption (Harvey 1966; Hurley and Brannon 1969). It is impossible, however, to determine whether these apparently different results reflect true differences in early feeding behavior or arbitrary differences in experimental conditions. For instance, the exact relations among emergence, maximum alevin wet weight, and yolk absorption vary as a function of the rearing environment, both among and within species (Heming 1982). Moreover, the onset of feeding can be influenced by the type of rearing substrate used (Leon 1975), premature exposure of fish to light (Kolgaev 1963), and water temperature (Braum 1967).

This study examined the transition from yolk absorption to active feeding in chinook salmon (*Oncorhynchus tshawytscha*), and the effect of temperature on that transition. Experiments were conducted to investigate the relationship between the timing of initial food presentation to alevins and their growth and survival at water temperatures from 6 to 12°C.

Materials and Methods

Egg Incubation

Gametes of chinook salmon were obtained from adults captured in the Campbell River, B.C. Mean hypural lengths ± 1 SD of female and male donors were 82.8 ± 4.6 cm (n = 12) and 82.6 ± 7.3 cm (n = 13), respectively. The eggs were fertilized and then placed in incubation trays (Heath Tecna-Plastics, Inc.) at densities of ~3000 per tray or 444 eggs/L. Mean egg weight at 10 h postfertilization was 490 ± 32 mg, of which 174 ± 15 mg constituted dry yolk (n = 60). Incubation trays were kept in darkness and were provided with 14.2 ± 0.7 L/min flows of dechlorinated city water from Nanaimo, B.C. Mean water temperatures during the incubation period were 5.9 ± 0.3, 7.9 ± 0.2, 9.8 ± 0.2, and 11.8 ± 0.3°C. Oxygen tensions in trays averaged 99.3 ± 3.6% of air saturation throughout the study. Incubation time to 50% hatching varied inversely with temperature, from 95 d (556 thermal units or tu) at 6°C, to 71 d (556 tu) at 8°C, to 55 d (534 tu) at 10°C, and to 44 d (520 tu) at 12°C. Thermal units were calculated from the relation,

$$(1)\quad \text{tu} = \sum \frac{\text{temperature}_i \times \text{hours at temperature}_i}{24},$$

where the time of fertilization and 0°C were the thresholds of time and temperature, respectively.

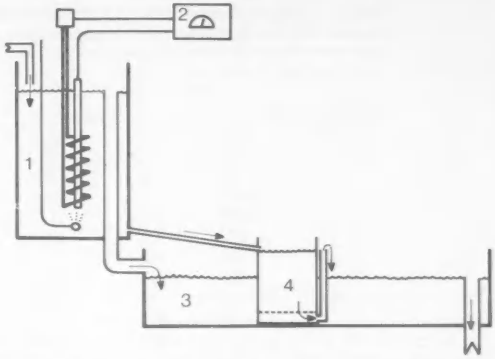

FIG. 1. Test unit used in feeding studies with chinook salmon: (1) headbox; (2) YSI Tele-thermometer model 73, with cooling and heating systems; (3) waterbath; (4) 1 of 20 fish containers. Arrows indicate direction of water flow. See text for details.

Test Apparatus and Conditions

Immediately after all eggs hatched at each temperature, the resulting alevins were transferred from the incubation trays to a test unit held at the same temperature. Each test unit consisted of 20 fish containers, a headbox, and a waterbath (Fig. 1). Fish containers were constructed of polyethylene and fiberglass, and had a volume of 5.8 L. Containers were covered with opaque tops which were removed when food presentation was initiated, as described below.

Initial loading density was 345 alevins per container, equivalent to ~30 g fish/L. Each container was provided with a 0.45 ± 0.02 L/min flow of dechlorinated Nanaimo city water filtered through 5 μm. Water temperatures during the feeding studies averaged 6.1 ± 0.3, 8.1 ± 0.2, 10.0 ± 0.1, and 11.9 ± 0.2°C. Average oxygen tensions in containers were 86.5 ± 9.6% of air saturation. Daily photoperiod during the feeding studies consisted of 11.4-h periods of light and dark, separated by 0.6-h morning and evening periods of simulated "twilight" at which time the intensity of illumination was gradually altered. Light intensity at the water surface of containers averaged 89.0 ± 4.8 lx during the light period and was <0.1 lx during the dark period.

Food Presentation

Alevins in the 20 containers at each temperature were assigned randomly among 10 feeding treatments, each treatment in duplicate. Treatments 1–9 differed in the time of initial food presentation; alevins in treatment 10 received no food (Table 1). Immediately prior to initial presentation of food, the tops of appropriate containers were removed permanently. As a result, initial exposure of alevins to food and light was almost simultaneous. This procedure models ponding of hatchery-reared salmon.

TABLE 1. Age of chinook salmon (days postfertilization) and thermal units (tu) when food presentation was initiated. Alevins in treatment 10 received no food.

Treatment no.	Temperature (°C)							
	6		8		10		12	
	Age	tu	Age	tu	Age	tu	Age	tu
1	124	734	96	761	78	766	64	751
2	143	848	109	870	90	885	73	855
3	152	903	117	926	97	955	76	897
4	155	922	121	958	99	975	79	929
5	160	952	125	991	102	1006	81	953
6	168	1002	132	1047	106	1045	87	1025
7	186	1112	146	1163	116	1146	95	1120
8	212	1269	157	1253	128	1266	104	1229
9	247	1483	182	1456	142	1408	119	1407

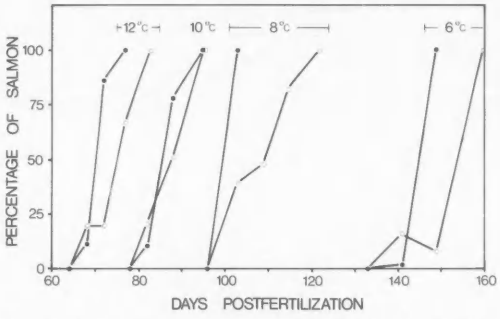

FIG. 2. Effect of temperature on occurrence of inflated gas bladders (●) and presence of ingested food (○) in chinook salmon. Each point represents data from 30 fish.

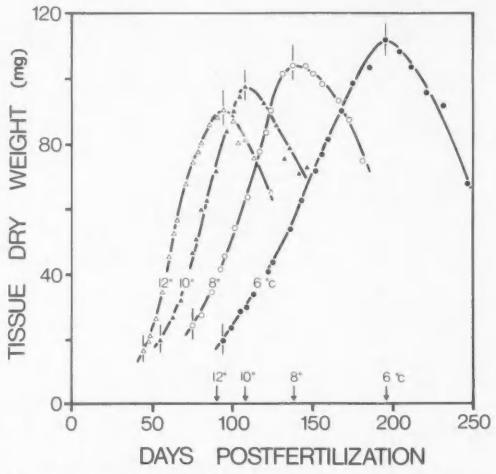

FIG. 3. Growth of nonfeeding chinook salmon at different temperatures (±95% confidence limits for selected means, $n = 30$). Arrows indicate the timing of maximum tissue weight (MTW).

Alevins were fed Formula II Oregon Moist Pellets (Moore-Clark Co.). Pellet size, daily ration, and frequency of food presentation were determined from a feeding chart available from the manufacturer. The only deviation from this chart was made during a period of 78 tu immediately following initial food presentation when Starter Mash (Moore-Clark Co.) was included in the diet. During the first two-thirds of this period, the proportion of mash in the diet was reduced in successive 13-tu intervals from 100%, to 75, to 50, and to 25%, and fish were fed at $\frac{1}{2}$-h intervals. During the latter third of this period, mash was included as 10% of the daily ration and fish were fed at 1-h intervals.

SAMPLING

Sampling from treatment 10 commenced when alevins were transferred to the fish containers and was discontinued at complete yolk absorption. Sampling from treatments 1–9 commenced immediately prior to initial presentation of food and was discontinued at 1634 tu postfertilization, about 178 tu after complete yolk absorption.

Sampled fish were anesthetized with tricaine methane sulphonate (MS222) and then preserved in 5% neutral formalin for at least 80 d. After preservation, alevins were blotted to remove excess water and weighed to the nearest 1.0 mg. Alevin fork length was measured to the nearest 0.5 mm. The pellet of crystalized yolk was dissected from the body of each fish and each of these components was weighed. In the present study, these components are referred to as yolk and tissue, respectively. Dry weights also were determined for each component after desiccation at 60°C for 48 h. Condition of the gas bladder (inflated or uninflated) and presence of food particles in the alimentary tract were recorded during separation of yolk and tissue. Wet and dry weights of ingested material were measured. Weights of ingested material were subtracted from alevin (tissue plus yolk) and tissue weights to compensate for the presence of ingested material. Only corrected alevin and tissue weights were used in subsequent analyses.

Development of chinook alevins was monitored by four methods: the developmental stages of Vernier (1969); the developmental index (k_D) of Bams (1970) ($k_D = 10 \cdot$alevin

TABLE 2. Effect of temperature on the age, weight, yolk reserves, developmental index (k_D), developmental stage and thermal units (tu) of nonfeeding chinook salmon when maximum alevin wet weight (MAWW) was reached (mean ± 95% confidence limits, n = 30).

Temp. (°C)	Age (d)	tu	Maximum alevin wet weight (mg)	Yolk dry weight (mg)	k_D	Developmental stage
6	186	1112	687 (666−709)	16 (14−18)	2.17±0.02	38
8	138	1099	673 (642−706)	17 (15−19)	2.18±0.02	38
10	106	1045	656 (633−680)	26 (23−29)	2.20±0.02	37
12	82	966	600 (580−620)	51 (46−57)	2.24±0.03	36

wet weight$^{0.333}$/alevin length); amount of dry yolk present; thermal units (Wallich 1901). Since Vernier (1969) classified development only as far as stage 37 (button-up), an additional stage (stage 38) was created to describe development between stage 37 and complete yolk absorption.

DATA ANALYSIS AND PRESENTATION

Standard treatment of data included Bartlett's test for homogeneity of variances, analysis of variance, and Duncan's multiple-range test. Differences present at the 5% level of probability ($P \leq 0.05$) were judged to be significant. As no significant differences were detected between data from duplicate containers, those data were pooled in subsequent comparisons. In analyses of weights and lengths, a $\log_e$ transformation was used to stabilize variances and to normalize the data distribution. Means of transformed data are reported as geometric means (means of transformed data back-transformed to their original scale) with their 95% confidence limits. Confidence limits are asymmetrical about geometric means and so are presented individually in parentheses after each mean. All other data are reported as arithmetic means ± their 95% confidence limits. Specific growth rates were calculated by multiplying the slope of the regressions of tissue dry weight ($\log_e$) with time, by 100 (Brett 1979).

Results

COMMENCEMENT OF FEEDING

Temperature had no effect on the stage of development at which chinook salmon were first capable of feeding. At all temperatures, alevins were at stage 34 when pursuit and ingestion of food particles was first observed, and when food particles first appeared in the alimentary tract of sampled fish. Thermal units at 50% ingestion, when 50% of sampled alevins showed the presence of ingested material, averaged 905 ± 14 among the four test temperatures. Alevins had a mean developmental index of 2.30 ± 0.03 and possessed 89 mg (86−94 mg) of dry yolk at 50% ingestion.

At each temperature, alevins began to ingest food at about the same time as they began to inflate their gas bladders (Fig. 2). Nonetheless, alevins were capable of ingesting food before inflating their gas bladders; 7.8% of sampled fish had done so.

Chinook salmon did not reach a "point-of-no-return," that is, a point after which food deprivation rendered the fish too weak to begin active feeding (Blaxter and Hempel 1963). All sampled alevins had ingested food in treatments in which initial food presentation occurred after the time of 50% ingestion (treatments 3−9 at each temperature). Initial feeding by alevins in these treatments was considered to have coincided with initial food presentation. In treatments in which fish were provided food before they were capable of feeding (treatments 1 and 2), initial feeding was considered to have coincided with 50% ingestion.

GROWTH

Chinook salmon hatched with 141 mg (136−146 mg) of dry yolk. The contribution to growth made by absorption of this internal reserve was assessed in nonfeeding fish (treatment 10). In the absence of external food, yolk absorption was accompanied by an initial increase and then a decrease in tissue dry weight (Fig. 3). Maximum tissue weight (MTW) was reached at 196 d postfertilization at 6°C, 146 d at 8°C, 106 d at 10°C, and 95 d at 12°C. The actual MTW achieved varied inversely with temperature (Fig. 3). Temperature had little effect on the developmental stage at which MTW was reached. Fish at MTW were at stage 38, had a mean developmental index of 2.14 ± 0.03, and possessed 14 mg (10−20 mg) of dry yolk. Thermal units at MTW averaged 1125 ± 58 among the test temperatures.

Alevin wet weight (tissue plus yolk) of nonfeeding fish also increased to a maximum and then declined during the yolk absorption period. These changes were related primarily to changes in the water content of alevins, as relatively dense yolk (51% water by weight) was converted into less dense tissues (84% water). Maximum alevin wet weight (MAWW) was reached before MTW at 6, 8, and 12°C; at 10°C, MAWW coincided with MTW. Temperature had a significant effect on the actual MAWW achieved, as well as on the developmental stage, k_D, yolk reserves, and thermal units at MAWW (Table 2). Nonfeeding fish reached MAWW at an earlier stage of development at higher temperatures. Fish that began feeding before reaching MAWW or MTW did not display distinct maxima.

Availability of food had no effect on yolk absorption (see Fig. 4A for representative results). Yolk absorption at each temperature was completed at the same time in all 10 treatments. Yolk reserves were exhausted more rapidly, however, at higher temperatures (Fig. 4B). Nonetheless, all alevins had reached a similar stage of development by the time yolk absorption was completed. Thermal units at complete yolk absorption averaged 1456 ± 22 among the test temperatures.

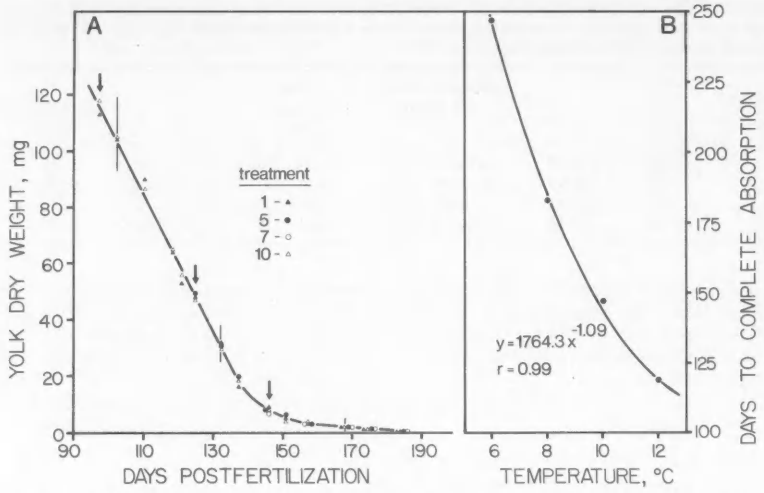

FIG. 4. Yolk absorption in chinook salmon: Panel A, effect of the time of initial food presentation (↓) on yolk absorption at 8°C (±95% confidence limits for selected means, $n = 30$); Panel B, effect of temperature on duration of the yolk absorption period (fertilization to complete yolk absorption).

The developmental index (k_D) of fish at complete yolk absorption was unaffected by temperature, but was influenced significantly by the timing of initial food presentation. Mean k_D values at complete yolk absorption decreased from 2.09 ± 0.04 in the earliest feeding fish (treatment 1) to 1.96 ± 0.02 in nonfeeding fish (treatment 10). These differences in k_D values did not appear to be related to actual variations in the developmental status of alevins at complete yolk absorption, but more likely reflected effects of the different feeding regimes on relative condition of the fish.

At stage 37 (button-up) when the yolk sac was completely covered with chromatophores, alevins still retained 45 mg (38–53 mg) of dry yolk internally. Thermal units at stage 37 averaged 1041 ± 43 among the test temperatures.

Availability of food had no effect on growth until shortly before MAWW was reached (see Fig. 5A for representative results). Mean tissue weight of fish that began feeding before reaching MAWW (treatments 1–6 at 6 and 8°C, treatments 1–5 at 10 and 12°C) did not differ significantly from that of nonfeeding fish until ~40 tu prior to MAWW, regardless of the exact time of initial food presentation. Moreover, the timing of initial food presentation in these treatments had no significant effect on fish size at the termination of the study (Fig. 6). Final mean tissue weights in these treatments did not differ significantly from one another, independent of temperature or the time of initial food presentation. Averaged over the four temperatures, the tissue dry weight of fish that began feeding prior to MAWW was 666 mg (654–678 mg) at the termination of the study (1634 tu postfertilization).

Initial food presentation was followed by an almost immediate increase in growth when food presentation was delayed until after fish reached MAWW (treatments 7–9 at 6 and 8°C, treatments 6–9 at 10 and 12°C) (Fig. 5A). However, such delays in the time of initial food presentation significantly reduced the final size of chinook salmon (Fig. 6). Final mean tissue weights in these treatments decreased in direct relation to the period of food deprivation after MAWW.

Growth rates of actively feeding fish were unaffected by the timing of initial food presentation but increased significantly at higher temperatures (Fig. 5B) (Table 3). Thus, differences in final fish size were attributable primarily to an effect of the delay in initial food presentation on fish size when food was first provided, rather than on subsequent growth rate.

SURVIVAL

Survival was a function of temperature and the timing of initial food presentation with respect to MAWW (Fig. 7). At each temperature, survival was maximized in treatments in which food presentation had been initiated before MAWW was reached. Most deaths in these treatments were characterized by embryonic hydrocoel (also termed "blue-sac"), coagulation of yolk, congenital anomalies, and/or fungal growth on the gills. Mortality in these treatments at 6, 8, and 10°C averaged 1.6 ± 0.4%. Mortality in similar treatments at 12°C was significantly greater, averaging 10.8 ± 2.5%. Delaying initial food presentation until after MAWW was reached increased total mortality; the longer the delay, the greater the mortality (Fig. 7). Most deaths in treatments in which food presentation was initiated after MAWW were characterized by severe emaciation.

Discussion

The transition from yolk absorption to active feeding in chinook salmon can include a period of "precocious feeding" during which ingestion of food occurs but is of no apparent benefit to growth or survival. A similar period has been noted

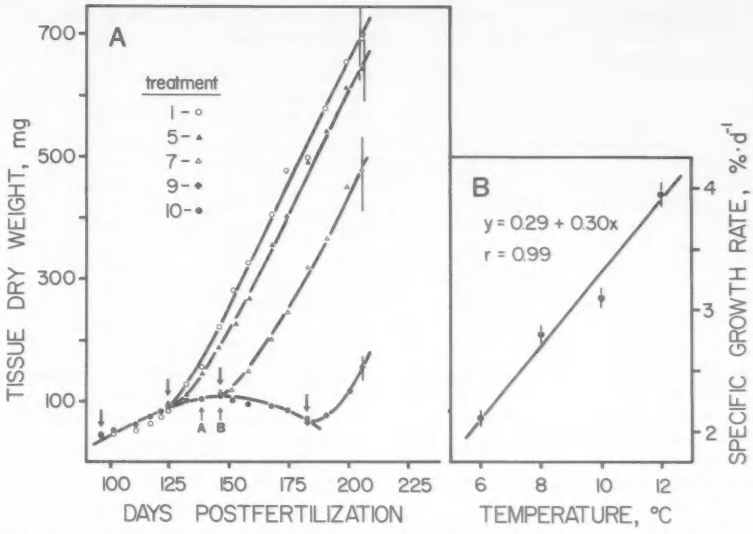

FIG. 5. Growth of chinook salmon: Panel A, effect of the time of initial food presentation (↓) on growth at 8°C (±95% confidence limits for selected means, $n = 30$). Occurrence of maximum alevin wet weight (A) and maximum tissue weight (B) in nonfeeding alevins are shown; Panel B, effect of temperature on the mean growth rate of feeding alevins (±95% confidence limits, $n = 9$).

in sockeye salmon (*Oncorhynchus nerka*) (Harvey 1966; Hurley and Brannon 1969). Hurley and Brannon (1969) considered precocious feeding to be a behavioral artifact arising from an encounter with food at an inappropriate time under abnormal gravel-free conditions. Although it is uncertain whether chinook salmon feed precociously in the natural environment, precocious feeding would be expected to occur in most cultural situations if food presentation were initiated prematurely. Precocious feeding is probably of little benefit to alevins. In the present study, ingestion of food prior to MAWW had no effect on subsequent fish size, growth rate, yolk absorption rate, or survival. Hurley and Brannon (1969) suggested that precocious feeding might have positive implications for later feeding behavior, presumably by allowing alevins the time to learn to feed efficiently before feeding is nutritionally necessary. However, Twongo and MacCrimmon (1976) were unable to demonstrate any relationship between feeding behavior and the time of first feeding in rainbow trout (*Salmo gairdneri*). Precocious feeding may in actuality be disadvantageous, as it has been associated with increased mortalities in some salmonids (Atkins 1905; Hurley and Brannon 1969). Ochiai et al. (1977) observed that premature feeding resulted in swallowing of food into the pneumatic duct during initial inflation of the gas bladder. Death ultimately ensued, apparently caused by bacterial infection of the gas bladder and other viscera. No evidence of such mortality was evident in our chinook salmon. Whether precocious feeding presents significant dangers to practical culture of salmon requires further study.

The potential duration of precocious feeding, that period between the time when alevins were first capable of ingesting

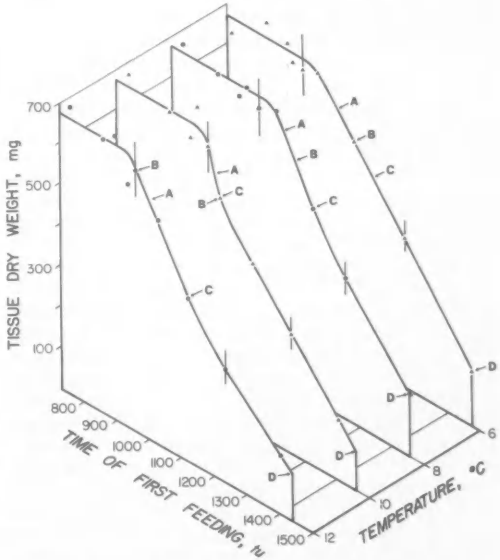

FIG. 6. Effect of the time of initial food presentation and temperature on the mean tissue weight of chinook salmon at 1634 thermal units (tu) (±95% confidence limits for selected means, $n = 30$). Occurrence of button-up (A), maximum alevin wet weight in nonfeeding fish (B), maximum tissue weight in nonfeeding fish (C), and complete yolk absorption (D) are shown.

TABLE 3. Effect of temperature and time of initial food presentation on the specific growth rate of chinook alevins (%/d ± 95% confidence limits). See text for method of calculation.

Treatment no.	Temperature (°C)			
	6	8	10	12
1	2.14±0.24	2.97±0.18	3.06±0.29	4.11±0.36
2	2.20±0.24	2.66±0.17	2.92±0.24	3.81±0.37
3	2.23±0.14	2.73±0.14	3.14±0.20	4.00±0.22
4	2.06±0.26	2.75±0.16	3.00±0.20	3.82±0.30
5	2.15±0.15	2.76±0.18	3.13±0.27	4.01±0.25
6	2.11±0.21	2.90±0.14	3.07±0.18	3.83±0.26
7	1.91±0.28	2.83±0.32	3.17±0.28	4.03±0.43
8	2.15±0.26	2.68±0.37	3.58±0.81	4.09±0.45
9	2.49±2.11	3.02±0.47	3.71±0.95	3.61±1.43

food and the time when feeding first became a significant benefit to growth and survival, varied inversely with temperature because of a temperature-dependent shift in the time of initial food utilization. Ingestion of food began at about 905 tu postfertilization at each temperature. The onset of ingestive behavior at that time was related to the disappearance of an epithelial occlusion of the alimentary tract anterior to the pneumatic duct (T. A. Heming unpublished data), similar to the epithelial plug present in immature *Salmo* spp. (Battle 1942; Prakash 1961; Twongo and MacCrimmon 1977). Utilization of food for growth did not occur until sometime later, however, and began earlier in development at higher temperatures. This probably was related to effects of temperature on the production and/or action of digestive enzymes. Braum (1967) reported that larvae of whitefish (*Coregonus warmanni*) also begin to utilize food earlier in development at higher temperatures.

Chinook salmon at each temperature in the present study began to ingest food and inflate their gas bladders at about the same time. On the basis of similar results at 8°C, Twongo and MacCrimmon (1976) concluded that rainbow trout normally begin feeding only after inflating their gas bladders. This is not necessarily the case, however, in chinook salmon. Wild salmonids inflate their gas bladders after emerging from the gravel redd by swallowing air through the pneumatic duct into the gas bladder (Tait 1960; Bams 1969). Thus, inflation of the gas bladder and the onset of feeding are ultimately linked to the disappearance of the epithelial occlusion of the alimentary tract. Nonetheless, initial feeding need not be dependent upon gas bladder inflation or emergence. In the present study, a small but significant proportion of alevins began to feed before inflating their gas bladders. Timing of 50% emergence of chinook salmon from artificial redds coincides with attainment of MAWW (Heming 1982). Considering that emergence involves a transitional period during which alevins repeatedly enter and leave the gravel (White 1915; Stuart 1953) and that chinook salmon are capable of both ingesting and utilizing food before MAWW is reached, chinook salmon probably are able to feed successfully before emergence is completed. This is consistent with field evidence which suggests that Pacific salmon (*Oncorhynchus* spp.) normally begin feeding before emerging from the gravel redd (Stuart 1953; Dill 1967; Thomas and Shelton 1968; Simonova 1972; Smirnov 1975). Such early feeding may explain the increased survival of wild migrating alevins when compared with unfed hatchery releases (Thomas and Shelton 1968).

Chinook salmon were capable of utilizing food to supplement nutrients made available by yolk absorption. Chinook salmon differ in this respect from sockeye salmon, in which feeding has no effect on growth until after all yolk reserves are exhausted (Harvey 1966; Hurley and Brannon 1969). Utilization of food before yolk absorption is completed would in effect decrease the nutritional requirements demanded of yolk. However, active feeding had no effect on yolk absorption in chinook salmon. This supports the proposal of Bams (1969) that at any given temperature the rate of yolk absorption is independent of variations in metabolic demand. This implies that increases or decreases in metabolic demands are not compensated for by parallel variations in the rate of yolk absorption but rather decrease or increase the resources available for growth. Feeding has been found to slow the rate of yolk absorption, however, in larvae of the California grunion (*Leuresthes tenuis*) (May 1971), tilapia (*Tilapia sparmanni*) (Ishibashi 1974), and Kuben inconnu (*Stenodus leucichthys nelma*) (Bogdanova 1977). Such a relationship between feeding and the rate of yolk absorption might increase the survival potential of larvae that depend upon a fluctuating or patchy supply of food, by enabling larvae to retain their yolk reserves when external sources of food are available to counter possible future shortages of food.

The effect of feeding on the developmental index (k_D) of alevins raises questions as to the general validity of using k_D as an indicator of developmental status, especially in situations in which feeding is neither monitored nor controlled. For this reason, considerable caution should be exercised when k_D values alone are used to monitor development, or when comparing k_D values between different species or experimental circumstances.

OPTIMUM TIME TO INITIATE FOOD PRESENTATION

In terms of maximal biomass production, there is no one distinct optimum time to begin food presentation to chinook salmon. Instead, the time of initial food presentation and temperature interact to create a zone of optimum feeding, within which the initiation of food presentation maximizes biomass production regardless of the exact time of initial food presentation or temperature (Fig. 8). Clearly, it would be of

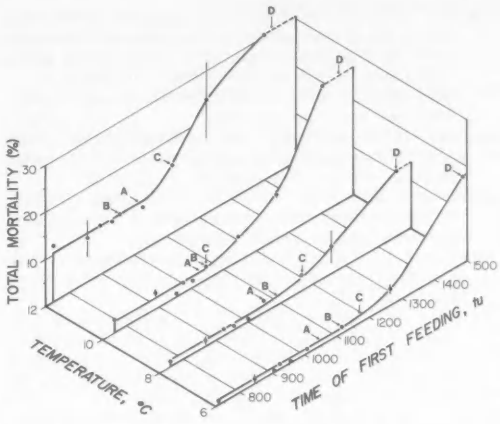

FIG. 7. Effect of the time of initial food presentation and temperature on the mortality in chinook salmon at 1634 thermal units (tu) (± 2 SE for selected means, $n = 2$). Rest of legend as in Fig. 6.

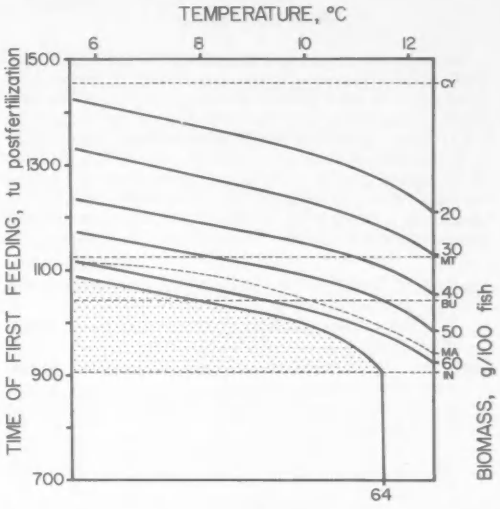

FIG. 8. Effect of the time of initial food presentation and temperature on the biomass expected from 100 newly hatched chinook salmon at 1634 thermal units (tu) postfertilization. Solid lines are estimated isopleths of biomass. Broken lines indicate occurrence of maximum alevin wet weight in nonfeeding fish (MA), and the mean time of 50% ingestion (IN), maximum tissue weight in nonfeeding fish (MT), button-up (BU), and complete yolk absorption (CY). The stippled region is the best estimate of the optimum feeding zone, within which biomass production was maximized.

little benefit to initiate food presentation before 905 tu, the point at which 50% of alevins were first able to ingest food. On the other hand, biomass was reduced when initial food presentation was delayed until alevins reached MAWW, MTW, or complete yolk absorption (Fig. 8). Initiation of food presentation at stage 37 (button-up) reduced biomass production at higher temperatures (10 and 12°C), but maximized biomass production at lower temperatures. This is consistent with the recommendation of MacCrimmon and Twongo (1980) that rainbow trout at 8°C should be fed from "button-up." On the other hand, Brown and Buck (1939) found no correlation between "button-up" and the onset of feeding in rainbow trout, brown trout (*Salmo trutta*), cutthroat trout (*Salmo clarki*), brook trout (*Salvelinus fontinalis*), or Arctic grayling (*Thymallus arcticus*) reared at 8 to 11°C. Biomass was maximized at all temperatures in the present study by initiating food presentation before alevins attained MAWW. This relationship between MAWW and the optimum time to initiate food presentation appears to hold true for a variety of fishes and under a variety of conditions. Reinterpretation of past studies indicates that growth and survival of chinook salmon (Palmer et al. 1951), Atlantic salmon (*Salmo salar*) (Marr 1965), and sockeye salmon (Harvey 1966) also were maximized when food presentation was initiated before MAWW was reached.

Conservative estimates of the point after which delays in initial food presentation reduced biomass production at each temperature were made from Fig. 8. These points (F, tu) did not coincide with any easily recognizable stage or event in development but were adequately described as,

$$F = 1201.1 - 20.3\,T \quad (2)$$

where T is temperature (°C) ($r = -0.979$). Comparison of the biomass produced at each temperature indicated that 12°C was supraoptimal for rearing chinook salmon (Fig. 8) because of greater alevin mortality at that temperature. This supports earlier observations that chinook salmon reared at 12°C experience increased mortality, hatch and emerge precociously, and are generally smaller than fish reared at lower temperatures (Heming 1982).

Easily discernible characteristics of chinook salmon within the optimum feeding zone included k_D values in the range of 2.2−2.3, T/A values (ratio of tissue wet weight to alevin wet weight) between 79 and 84%, and yolk dry weights between 40 and 70 mg. However, in situations where k_D or T/A values are used as indicators of the time of initial food presentation, it must be remembered that fish decrease in length and increase in weight during preservation in formalin; the magnitude of these changes is a function of the duration of preservation, the preservative concentration, and the tissue/yolk composition of preserved alevins (Heming and Preston 1981). In general, preservation in formalin decreases k_D values and increases T/A values.

The exact boundaries of the optimum feeding zone are probably specific to the species and rearing conditions, and so equation 1 and Fig. 8 should be used only to approximate the optimum time to begin providing food to alevins. In production facilities, presentation of food should be initiated somewhat earlier than the time generated in equation 1. Such a practice would compensate for variations in the developmental status of alevins arising from differences in the timing of gamete collection and fertilization, or from variations in the incubation conditions of individual fish.

Acknowledgments

This study is based on a thesis submitted by T.A. Heming in partial fulfillment of requirements for the degree Master of Science at the University of Victoria, and was conducted at the Pacific Biological Station. The advice and assistance of the P. B. S. staff, in particular F. P. J. Velsen and R. P. Preston, is gratefully acknowledged. Workers at the Quinsam Hatchery, Campbell River, B.C., are thanked for their assistance in obtaining the experimental fish. This research was supported by the Department of Fisheries and Oceans, and by scholarships awarded to T. A. Heming by the former National Research Council of Canada.

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Cyclopoid Predation on Lake Michigan Fish Larvae[1]

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HARTIG, J. H., D. J. JUDE, AND M. S. EVANS. 1982. Cyclopoid predation on Lake Michigan fish larvae. Can. J. Fish. Aquat. Sci. 39: 1563–1568.

Cyclopoid predation on fish larvae, as evidenced by copepods attached to larvae in field collections, was quantitatively investigated during 1975–76 in southeastern Lake Michigan. Although six species of fish larvae were collected, predation occurred primarily (98%) on alewife (*Alosa pseudoharengus*); 2% of the predators were attached to spottail shiner (*Notropis hudsonius*) larvae. No cyclopoids were observed on rainbow smelt (*Osmerus mordax*), yellow perch (*Perca flavescens*), common carp (*Cyprinus carpio*), or sculpin (*Cottus* spp.) larvae. Most alewife larvae attacked were 3–8 mm long; older larvae and larvae of more robust species, such as yellow perch and spottail shiner, are apparently immune to such predation. Most predation (99%) occurred in July when alewife larvae were numerous and cyclopoids abundant. Fish larvae with attached copepods were found only in night collections. Most cyclopoid predators (99%) were adult female *Diacyclops thomasi* and *Acanthocyclops vernalis*. Other predaceous species of zooplankton, that occurred in close temporal and spatial proximity to fish larvae, apparently were not predaceous on these organisms.

Key words: *Alosa pseudoharengus* larvae, *Notropis hudsonius* larvae, *Diacyclops thomasi*, *Acanthocyclops vernalis*, cyclopoid predation, Lake Michigan

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Une étude quantitative de la prédation par les cyclopoïdes sur les larves de poisson, telle que démontrée par les copépodes fixés sur les larves dans les collections sur le terrain, a été menée en 1975–76 dans le sud-est du lac Michigan. Bien que les larves de six espèces de poissons aient été capturées, la prédation se trouvait surtout (98%) chez le gaspareau (*Alosa pseudoharengus*); alors que 2% des prédateurs étaient fixés aux larves de queue à tache noire (*Notropis hudsonius*). Aucun cyclopoïde n'a été observé sur les larves d'éperlan arc-en-ciel (*Osmerus mordax*), de perchaude (*Perca flavescens*), de carpe commune (*Cyprinus carpio*) et de chabots (*Cottus* spp.). Les larves de gaspareau attaquées mesuraient, pour la plupart, 3–8 mm de long; les larves plus âgées ainsi que celles des espèces plus robustes, telles que la perchaude et le queue à tache noire, ne sont apparemment pas touchées par une telle prédation. Elle se produit surtout en juillet (99%), quand les larves de gaspareau sont nombreuses et le cyclopoïdes abondants. C'est seulement dans les collections de nuit qu'on a trouvé des larves de poisson sur lesquelles étaient fixés des copépodes. La plupart des cyclopoïdes prédateurs (99%) étaient des femelles adultes de *Diacyclops thomasi* et *Acanthocyclops vernalis*. D'autres espèces zooplanctoniques prédatrices se trouvant à proximité, dans le temps et dans l'espace, des larves de poisson ne semblent pas les attaquer.

Received February 10, 1982
Accepted August 13, 1982

Reçu le 10 février 1982
Accepté le 13 août 1982

COMMUNITY structure and composition in aquatic ecosystems can be markedly affected by predator–prey interactions (Brooks and Dodson 1965). Cyclopoids are important freshwater invertebrate predators in temperate lakes (Kerfoot 1977). Many investigations emphasize only zooplankton prey in cyclopoid predation (Smyly 1970; Anderson 1970; Confer 1971; Brandl and Fernando 1974); however, cyclopoids are also known to attack prey much larger than themselves (Hutchinson 1967), including fish larvae (Davis 1959; Fabian 1960; Lillelund 1966; Sukhanova 1965; Lillelund and Lasker 1971). Year-class strength in fish is mainly controlled by mortality in the egg and larvae stages, so it is vital to understand how factors such as invertebrate predation affect these life history stages. We have documented a significant incidence of cyclopoid predation on fish larvae in southeastern Lake Michigan when cyclopoids and small fish larvae

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Printed in Canada (J6810)
Imprimé au Canada (J6810)

attained spatial and temporal proximity.

Samples were collected during 1975–76 in the nearshore region of southeastern Lake Michigan (near Bridgman, Michigan) as part of an environmental surveillance study at the D. C. Cook Nuclear Power Plant site. Baseline data on inshore Lake Michigan fish populations (Jude et al. 1975, 1979) and zooplankton distribution studies (Evans 1975; Evans et al. 1978) give background information on these respective biota in the study area.

Methods

Fish larvae, arbitrarily defined as any fish less than 25.4 mm total length, were collected using a 0.5-m-diameter, 363-μm mesh net equipped with a calibrated flowmeter. Fish larvae were sampled at nine stations once a month (April–November) during the day (usually from 14:00 to about 2 h before sunset) and night (usually 1 h after sunset to about 03:00). Two transects were established ~8 km apart; two beach stations were located at 1 m on one transect, whereas one was located on the other. On each transect, stations were also located at 6 m (stations C, G), 9 m (stations D, H), and 21 m. At beach stations, duplicate surface tow samples were obtained by hand-towing the net against the current for 60 m parallel to shore. At open-water stations, 4-min horizontal tows parallel to shore were conducted from the University of Michigan's R/V Mysis at a speed of 3.2 to 6.4 km/h. Samples were collected at 2-m depth intervals at 6-m stations (four depths) and 9-m stations (five depths), and at 0.5, 8, 14, and 20 m at 21-m stations. Samples were immediately preserved in 10% formaldehyde.

Fish larvae were removed from samples with the aid of a binocular microscope and a lighted sorting chamber (Dorr 1974). Larvae were identified and total length (TL) measured to the nearest 0.1 mm. Length–frequency histograms for fish larvae were generated using 0.5-mm intervals.

Cyclopoid attachment to fish larvae was recorded when found. Species, developmental stage of zooplankter, number of cyclopoids attached, and attachment location on larvae were recorded.

Zooplankton were sampled over a 250-km^2 survey grid in Lake Michigan. Major surveys consisted of 30 stations and were conducted in April, July, and October. Short surveys consisting of 14 stations were conducted in May, June, August, and September. (For details see Evans et al. 1978.)

Zooplankton were also sampled once a month from the intake and discharge forebays of the power plant; intakes are located at approximately the 9-m contour. A diaphragm pump drew water from the forebay through a 7.6-cm-diameter pipe which discharged water and zooplankton into a 30-cm-diameter net (156-μm mesh) suspended in a barrel of water to minimize damage to zooplankton. Water flowed from the bottom of the barrel through an outlet pipe equipped with a flowmeter. Duplicate samples were collected from each forebay at noon, sunrise, sunset, and midnight. All samples were immediately preserved with a sugar–formaldehyde solution.

In the laboratory, two of the three field survey samples from each station and all entrainment samples were subdivided as many times as necessary in a Folsom plankton splitter to give two subsamples of 350–500 organisms each. Zooplankton were identified to species (adult copepods, cladocerans), genus (immature copepodites), or suborder (nauplii), and enumerated. A third subsample of 700–1000 zooplankters was examined for rare zooplankton (less than 40 animals in the first two subsample counts).

Results

Predaceous cyclopoids *Acanthocyclops vernalis* and *Diacyclops thomasi* (= *Cyclops bicuspidatus thomasi*) (Robertson and Gannon 1981) were found attached to alewife (*Alosa pseudoharengus*) and spottail shiner (*Notropis hudsonius*) larvae. Up to three predaceous cyclopoids were found attached to a single larva (Fig. 1) and in some cases up to one-third of a fish larva apparently had been consumed. Ninety-eight percent of the observed cyclopoid predation occurred on alewife larvae; all predation was recorded from night samples. Ninety-nine percent of the observed cyclopoid predation on fish larvae was reported from July 1975, 1976 collections. In July 1975, 20 alewife larvae were preyed on at night at 6- and 9-m stations, whereas in July 1976, 76 were preyed on, which represented, respectively, 1.1 and 3.7% of the total number of alewife larvae (1766 and 2073) collected at that time. In night collections from August 1975, 1 of 409 alewife larvae collected was attacked. Maximum incidence of cyclopoid predation in a given tow was 16%, which occurred in a surface sample collected during July 1976 at 6-m station C (Fig. 2).

Cyclopoid predation observed in July 1975 was exclusively by *A. vernalis*, whereas in July 1976 both *A. vernalis* and *D. thomasi* were involved. Of the cyclopoids exhibiting predation on fish larvae, 99% were adult females. Only one *D. thomasi* at the copepodid V stage was found attached to an alewife larva. Other zooplankton predators including *Epischura lacustris*, *Polyphemus pediculus*, and *Leptodora kindtii*, although observed in the nearshore plankton, apparently did not attack fish larvae.

In the Cook survey area, adult *D. thomasi* tended to occur in low abundances in the shallowest areas of the zooplankton survey grid (6-m contour) and to increase in abundance with increasing water depth from spring to autumn (Evans et al. 1978). Entrainment data (Fig. 3) showed that adult *D. thomasi* were common in day and night samples indicating no diel vertical migration pattern. However, despite their presence in similar numbers in day and night samples, predation only occurred on fish larvae at night. Fewer adult *D. thomasi* were collected in July 1975 than in July 1976.

Acanthocyclops vernalis is not a numerically important constituent of zooplankton in the Cook survey area (Evans et al. 1978). Very few were found in daytime vertical hauls during the period of maximum *A. vernalis* predation (July 1975, 1976). Entrainment data documented that this species entered the plankton at night (Fig. 3) from the sediment–water interface (Evans and Stewart 1977) and undoubtedly occurred in greater numbers than suggested by data generated from day plankton collections.

During 1975–76, peak abundance of alewife larvae at 6- and 9-m stations was observed during July (Fig. 4). Peak

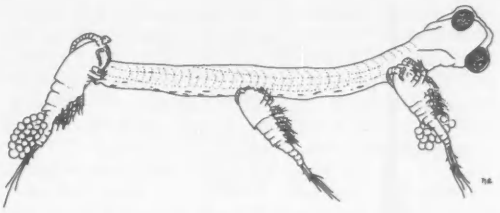

FIG. 1. Three *Acanthocyclops vernalis* attached to an 8-mm alewife (*Alosa pseudoharengus*) larva collected at night during July, 1976. Drawing is from an actual larva collected from Lake Michigan.

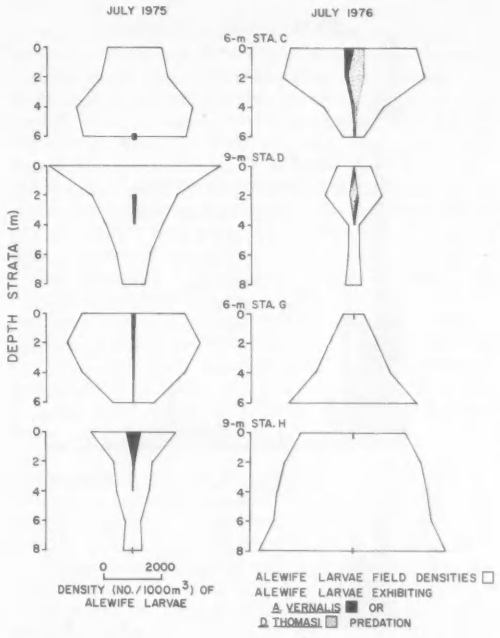

FIG. 2. Field densities of alewife larvae (line width) with density of those attacked by two predaceous copepod species by depth stratum and station during July 1975–76 in southeastern Lake Michigan.

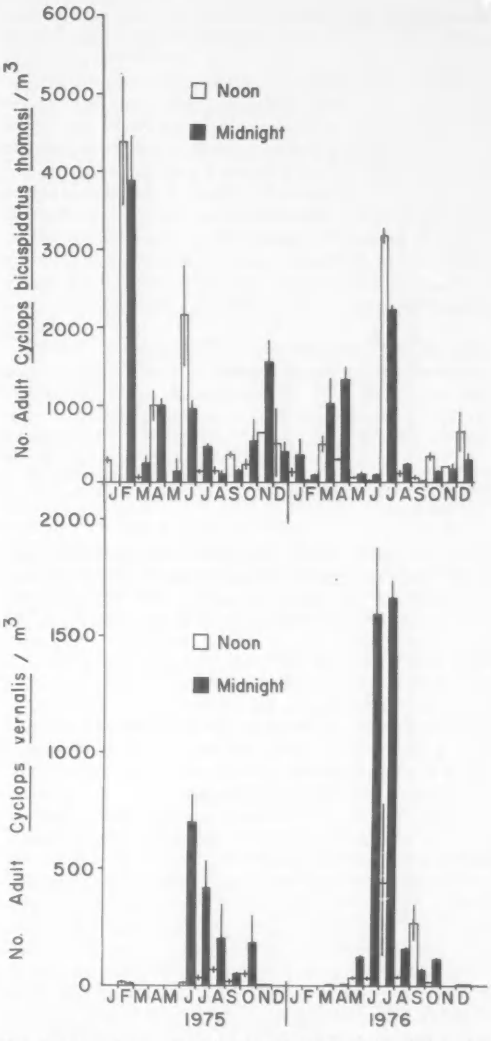
No. Adult Cyclops bicuspidatus thomasi / m³
Noon
Midnight
J F M A M J J A S O N D J F M A M J J A S O N D
No. Adult Cyclops vernalis / m³
Noon
Midnight
J F M A M J J A S O N D J F M A M J J A S O N D
1975
1976

FIG. 3. Abundance of adult *Diacyclops thomasi* and *Acanthocyclops vernalis* in entrainment samples collected monthly at noon and midnight from southeastern Lake Michigan, 1975–76. Water was drawn from the 9-m contour. Vertical lines are one standard error.

alewife larvae abundance at beach stations occurred in June, while only a few were found at 21-m stations; alewife larvae were never observed in the hypolimnion.

Only two incidences of spottail larvae predation (beach stations, July 1976) were recorded. No cyclopoid predation was recorded on other species of larval fish, despite the collection during April–August 1975–76 of 85 common carp (*Cyprinus carpio*), 109 yellow perch (*Perca flavescens*), 67 sculpin (*Cottus* spp.), and 89 rainbow smelt (*Osmerus mordax*) larvae.

Although there was a wide range (3–25 mm) of sizes of alewife larvae present in our field collections (Fig. 5), only larvae within a narrow length range (3–8 mm), with the exception of three larvae >20 mm, were attacked. Mean length of larvae preyed on in 1975 was 5.3 mm (n = 20), while in 1976 mean length was 5.9 mm (n = 76). Newly hatched alewives ranged from 3 to 5 mm.

Frequency of attachment of predaceous cyclopoids on fish larvae was not random. Of 116 sites where cyclopoids successfully attached, 86 (74%) were on the terminal ends of the larvae (head, 31; postanal finfold, 44; postanal body, 11), 25

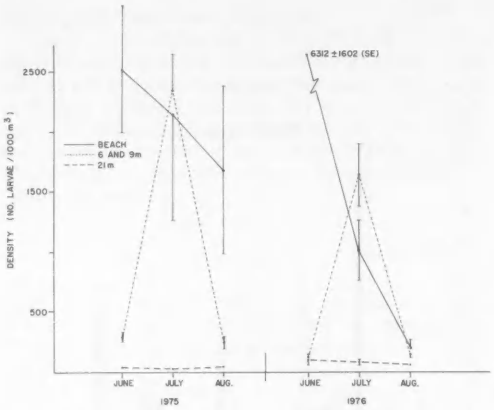

FIG. 4. Density of alewife larvae at beach (n = 2), 6- and 9-m stations (n = 36), and 21-m stations (n = 16) pooled over day and night (two), station (two–four), and replicate (two) or depth (four or five) in southeastern Lake Michigan, 1975–76. Vertical bars are one standard error.

(21%) were on the preanal body surface (from behind the head to the vent), and 2 (2%) were on the preanal finfold (located on the dorsal and ventral surfaces). Three alewives over 20 mm had copepods attached to pelvic, pectoral, and caudal fin tissue.

Discussion

Although many species of cyclopoids may exhibit selective feeding in particular habitats, most species are probably capable of feeding in several different ways on various foods, depending on circumstances (Monakov 1959). In our study, cyclopoid predation on fish larvae occurred when predaceous adult cyclopoids and susceptible young fish larvae attained temporal and spatial proximity.

In southeastern Lake Michigan, alewife spawn from June through August, with peak numbers occurring during warmest months, usually July–August (Jude et al. 1979). Immature and adult *D. thomasi* are common throughout the year, although the winter population tends to be dominated by immature copepodites (Torke 1975). *Acanthocyclops vernalis* is primarily a warmwater species occurring in highest numbers during summer (Gannon 1972). In our study, most cyclopoid predation on fish larvae occurred in July, with none in June, despite the high abundance of larvae. We attributed this to the scarcity of predaceous cyclopoids in the area. By August, reduced alewife hatching was occurring and numbers of predaceous cyclopoids were declining, resulting in the subsequent decline in occurrences of predation.

From laboratory observations of carnivorous copepods, Fryer (1957) notes that chance plays a considerable part in the actual encountering of prey. Actual prey selection by predatory copepods appears to be based on several factors. Experimental studies on predaceous copepods have shown that *A. vernalis* and *D. thomasi* will attack zooplankton species of various sizes (Anderson 1970). Laboratory experiments of

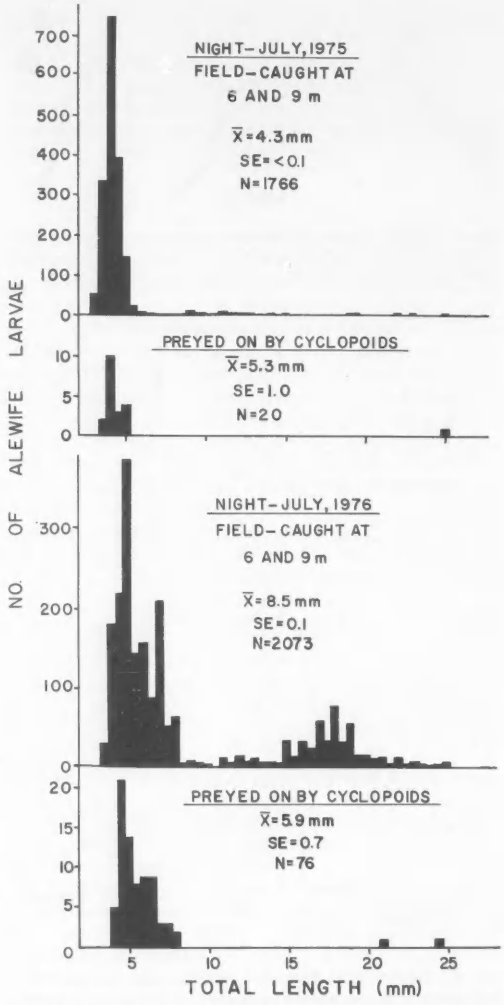

FIG. 5. Length–frequency distributions of alewife larvae collected during nighttime field sampling in July 1975–76 and those preyed on by *Diacyclops thomasi* and *Acanthocyclops vernalis*. All larvae were collected at 6- and 9-m stations. $\bar{x}$ = mean, SE = standard error, and n = sample size.

prey selection by *A. vernalis* have demonstrated that body shape, carapace hardness, and mode of swimming were major influences on the selective process (Li and Li 1979). Zooplankton that were slow swimmers and had soft bodies and narrow body shapes with few or no projections were readily selected. Regarding prey selection by cyclopoids, Anderson (1970) states that prey species swimming speed or strength are probably more important than size.

We found that predominantly small alewife larvae were

preyed upon by *A. vernalis* and *D. thomasi*. Alewife larvae are the most-abundant fish larvae during the summer in the inshore waters of southeastern Lake Michigan (Jude et al. 1979); they are small (3–5 mm at hatching), soft-bodied, relatively slow swimmers, and probably planktonic for a short period as has been found for other clupeid larvae (Graham et al. 1972). As alewife larvae mature, they grow larger, swim stronger, and develop harder body coverings.

Spottail shiner is the second most-abundant larval fish in this area of southeastern Lake Michigan, with densities in the beach zone often comparable to alewife densities in the inshore zone (Jude et al. 1979). Adults spawn during June and larvae are concentrated on the bottom from 3 m to shore. Despite the presence of large numbers of these larvae, only two incidences of cylopoid predation were observed. Probable reasons for this low level of predation are: (1) spottail larvae are robust, extremely mobile larvae and consequently effective in escaping attacks by predaceous cyclopoids; (2) predaceous cyclopoids are not common in the beach zone during maximum abundance of the vulnerable newly hatched larvae, and (3) by July spottails are large, agile, and have a hard dermal covering. Lack of predation on other species of fish larvae is partially due to temporal and spatial asynchrony of predator and prey, but is also due to the more robust nature of yellow perch, carp, and sculpin larvae which affords them some immunity to predation.

The hypothesis of cyclopoids preying on small, soft-bodied, slow-swimming prey is supported by laboratory experiments (Lillelund and Lasker 1971) concerning predation by marine copepods on fish larvae. They found that increased swimming and escape ability developed as anchovy larvae became older. Theilacker and Lasker (1974) in their laboratory studies noted that as marine larvae age they become more active and that increasing larval fish activity correlated well with a decreasing ability of shrimp, *Euphausia pacifica*, to capture larval anchovies. These authors concluded that abundance of euphausids and other predators may be a factor in determining the patchiness of fish larvae in the ocean. In addition, Wickstead (1959) noted from field observations on a predatory marine copepod that its chaetognath prey were more flexible (soft-bodied) than most chaetognath species in the Singapore area, and apparently were more easily grasped by the copepod.

Our results also show that most successful attacks originated from a lateral position rather than dorsally or ventrally (one was attached to the vent). As fish larvae mature and develop harder body coverings, we expected that the number of successful attacks would be fewer and confined to the softest, most accessible areas (fin tissue). Our results supported this because the only three fish larvae over 20 mm that were attacked had cyclopods attached to pelvic, pectoral, and caudal fin tissue.

In our study on fish larvae, predation was found only in night collections, with an indication of greater predation near the surface, rather than near bottom. *Acanthocyclops vernalis* undergoes nocturnal vertical migration. Feeding near the surface at night by predaceous copepods may have adaptive significance, because copepods avoid being eaten by visual-feeding fish during the day (Wetzel 1975). Avoidance of predatory copepods by larval fish using their vision during the day may explain the lack of any daytime predation by *D. thomasi*, despite its presence high in the water column during the day.

Although percentages of the total number of larvae preyed upon in this study were low, predation can be occasionally significant, because up to 16% of the larvae in one sample was attacked. Also, larvae are attacked each night and are clearly vulnerable over at least a month. Certainly those larvae attacked must be adversely affected, as we have found severely deformed larvae at the point of attachment and up to one-third of the tail of one larva was missing. Based on laboratory experiments and pond observations, Sukhanova (1965) determined that *A. vernalis* predation significantly affected survival rates of white carp larvae (*Hypopthalmichthys* spp.) cultured in Asia.

Lastly, even though it has been suggested that abnormal feeding may occur among copepods immediately after capture (Wickstead 1959), our estimates of incidence of predation may be low when the work of Lillelund and Lasker (1971) is considered. They found that marine copepods detached from larval fish upon addition of 3% formaldehyde.

In this study, one or more incidences of cyclopoid predation on fish larvae were found in 33 fish larvae tows conducted at various strata and localities in Lake Michigan during 1975–76. Because alewife larvae did not occur in the lake in at least present-day numbers until the 1960s (Brown 1972), the amount and intensity of predation is a recent development and an example of a predator availing itself of suitable available prey. Cyclopoid predation occurred consistently in 2 years, primarily in July, and was only observed in night collections. The amount and frequency of larval fish damage from predaceous cyclopoids suggest that invertebrate predation can be a significant mortality factor at some locations during certain times of the year.

Acknowledgments

This study was partially funded from a grant to J. C. Ayers from the Indiana and Michigan Power Company. We thank Bruce Pollard for his earlier work on the project, Nancy Auer for the alewife drawing, Sheryl Corey and Girard Lillie for drafting, and Frank Tesar, Al Beeton, Richard Stemberger, and Jim Bowers for critical reviews. Judy Li shared her thesis research results before their publication.

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Predatory Behavior and Prey Selectivity of the Pelagic Water Mite *Piona constricta*

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RIESSEN, H. P. 1982. Predatory behavior and prey selectivity of the pelagic water mite *Piona constricta*. Can. J. Fish. Aquat. Sci. 39: 1569–1579.

Predation by the pelagic water mite *Piona constricta* on the various components of the zooplankton community of Heney Lake, Quebec, Canada, was investigated through both direct observation and several series of feeding experiments. On encountering a prey item, the mite seized the organism with its four pairs of legs and pair of palps and tore a hole in the body wall with its chelicerae. The soft body tissues of the prey were predigested and the liquid nutriment was then drawn into the mouth. Pionid adults ate an average of 10–15 prey·mite^{-1}·d^{-1} and selected prey items as follows: *Bosmina* > *Daphnia*, *Ceriodaphnia* > *Chydorus* >> *Diaphanosoma*, *Diaptomus* > *Mesocyclops*. The nymphs ate only 2–3 prey·mite^{-1}·d^{-1} with *Bosmina* and *Chydorus* as the preferred items. The other cladocerans and the copepods were only rarely eaten. The pattern of prey selection for the adults and nymphs appeared to depend primarily on the escape response of the prey (their ability to detect an approaching mite and flee the area before being grabbed). Prey size and carapace thickness were of secondary importance.

Key words: water mites, *Piona constricta*; zooplankton, predator–prey interactions

RIESSEN, H. P. 1982. Predatory behavior and prey selectivity of the pelagic water mite *Piona constricta*. Can. J. Fish. Aquat. Sci. 39: 1569–1579.

L'observation directe ainsi que plusieurs séries d'expériences de nutrition ont servi dans l'étude de la prédation de la pionide aquatique pélagique *Piona constricta* sur les diverses composantes de la communauté zooplanctonique du lac Heney, au Québec (Canada). Lorsqu'elle rencontre une proie, la pionide la saisit dans ses quatre paires de pattes et sa paire de palpes et, à l'aide de ses chélicères, perfore la paroi du corps. Les tissus corporels mous de la proie sont pré-digérés et le nutriment liquide est ensuite aspiré dans la bouche. Les pionides adultes mangent en moyenne 10–15 proies·individu^{-1}·jour^{-1} et leur préférence est dans l'ordre suivant : *Bosmina* > *Daphnia*, *Ceriodaphnia* > *Chydorus* >> *Diaphanosoma*, *Diaptomus* > *Mesocyclops*. Les nymphes ne mangent que 2–3 proies·individu^{-1}·jour^{-1}, *Bosmina* et *Chydorus* étant les proies préférées. Les autres cladocères et copépodes sont rarement mangés. Les modalités de sélection des proies des adultes et des nymphes semblent dépendre surtout de la réaction d'échappement des proies (leur aptitude à détecter le prédateur qui s'approche et à fuir avant d'être saisies). La taille et l'épaisseur de la carapace des proies ont une importance secondaire.

Received February 17, 1982
Accepted July 15, 1982

Reçu le 17 février 1982
Accepté le 15 juillet 1982

THE carnivorous zooplankton community in the pelagic zone of lakes is composed of a variety of different types of invertebrates, including several species of cyclopoid and calanoid copepods, the cladoceran *Leptodora kindtii*, the rotifer *Asplanchna*, the midge larva *Chaoborus*, and the mysids *Mysis* and *Neomysis*. Predation by these animals can contribute significantly to mortality in their prey populations and be a very important force in structuring plankton communities (Dodson 1974a, b; Fedorenko 1975; Kerfoot 1975, 1977; O'Brien et al. 1979; Murtaugh 1981). Water mites are not generally considered a part of this association, as they are usually limited to the littoral and benthic areas of lakes, as well as ponds and streams (see Riessen 1982). Although mites have occasionally been found in plankton tows (see Hutchinson 1967; Riessen 1982), it has only been recently that their permanent presence and relatively high densities in the open-water plankton communities of certain lakes has been established and appreciated. Gliwicz and Biesiadka (1975) found *Piona limnetica* throughout the year in the pelagic zone of Madden Lake, Panama, at densities reaching 150/m^3. I have recently found equally high densities of the water mite *Piona constricta* (Wolcott) (Pionidae) in the pelagic plankton community of Heney Lake, Quebec, Canada. These mites reach densities exceeding 100/m^3 during much of

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Printed in Canada (J6822)
Imprimé au Canada (J6822)

the ice-free period of the year (Riessen 1982) and exhibit a distinct diel vertical migration typical of other zooplankton (Riessen 1980). In addition to *P. constricta*, several other species of water mites are often common (but never greatly abundant) in the Heney Lake plankton community: *Unionicola crassipes* (Unionicolidae); *Huitfeldtia rectipes* (Pionidae), and a few additional species of *Piona* (Riessen 1982).

Water mites have six distinct developmental stages, three of which (the larva, nymph, and adult) are active, whereas the others are quiescent (see Mitchell 1957; Smith 1976). The pelagic *P. constricta* of Heney Lake has a single generation per year: nymphs overwinter in the bottom sediments of the lake and emerge in the spring to transform (via the imagochrysalis stage) into adults; the eggs that are subsequently laid hatch into larvae which then metamorphose (via the nymphochrysalis stage) into a new generation of nymphs during the summer (Riessen 1981, 1982).

The larvae of most water mite species become ectoparasites on various aquatic insects [the larval stage of Heney Lake *P. constricta* is an exception (see Riessen 1981, 1982)], whereas the nymphs and adults are nearly always carnivorous (see Mitchell 1957; Smith 1976; Smith and Oliver 1976; Böttger 1976; Pieczyński 1976). The potential prey of the nymphs and adults includes cladocerans, copepods, ostracods, and insect larvae, depending upon the nature of the particular mite species (Motas 1928; Pieczyński 1976). Mites in the genus *Piona* are reported to be especially voracious predators (Wolcott 1902; Paveljeva and Zánkai 1971). Although there have been only a few investigations into the quantitative nature of water mite predation, several studies have concluded that these predators can have a significant impact on their prey populations (Pieczyński 1976; Gliwicz and Biesiadka 1975; Laird 1947; Paterson 1970). Predatory behavior, feeding rates, and prey selectivity of the pionid water mites (especially *P. constricta*) in the plankton community of Heney Lake are examined in this paper.

Methods

Predation experiments to determine feeding rates and prey selectivity of water mites were run for both adults and nymphs during the summers of 1978 and 1979. All adult pionid mites were collected directly from Heney Lake with a 110-μm mesh plankton net, whereas nymphs were either collected from the lake or raised from eggs. In all experiments with nymphs and one series with adults (Ad 4), only *P. constricta* was used (experiments run during 1979). The remaining experiments with adults contained pionids found in the limnoplankton at the time of the year collected (Ad 1: late June–August 1978; Ad 2, Ad 3, Ad 5: August–September 1978); these were mostly *P. constricta*, but a few individuals of one or two other species of *Piona* and *H. rectipes* were also present. There were no discernible differences in predatory behavior between *P. constricta* and these other species.

A variety of zooplankters found in Heney Lake were tested as potential prey: the cladocerans *Bosmina longirostris*, *Chydorus sphaericus*, *Daphnia pulex*, *D. galeata mendotae*, *Ceriodaphnia lacustris*, and *Diaphanosoma leuchtenbergianum*, and the copepods *Mesocyclops edax* and *Diaptomus* spp. Copepods used in the experiments were collected from the lake a few days prior to an experiment, whereas cladocerans were cultured from individuals captured previously. All prey were from Heney Lake, except for *Bosmina* which were cultured from individuals taken from Pink Lake, Quebec. Several prey in each experiment were measured (body length, excluding spines) with an ocular micrometer at 40× or 63×. Unless otherwise stated, the mean sizes (in millimetres) of prey used in the experiments were as follows: *Bosmina*, 0.35–0.40; *Chydorus*, 0.30–0.35; *Ceriodaphnia*, 0.55–0.60; *Diaphanosoma*, 0.70–0.75; *Daphnia pulex*, 0.85–1.00; *Diaptomus*, 0.85–0.90.

Predatory Behavior

Predatory interactions between water mites and their potential zooplankton prey were observed with and without the aid of magnification, and also filmed with a Bolex H16 motion picture camera at 20 frames/s using the methods of Strickler (1977). These interactions were analyzed in terms of the individual events of encounter, attack, capture, and ingestion (Gerritsen and Strickler 1977). The swimming speed of adult pionid mites was determined from frame-by-frame analysis of the films, in which the digitized coordinates of the mite on successive frames were used to calculate the distance moved (see Gerritsen 1978).

Predation Experiments

Five series of experiments had adults as predators (Ad 1–Ad 5), and five had nymphs (Ny 1–Ny 5); these are detailed below and in Table 1. Initial experimental prey densities were a very close approximation to total crustacean zooplankton densities in Heney Lake during the summer. Experimental chambers for Ad 1 were 500-mL Erlenmeyer flasks, except for one set of experiments with *Daphnia* spp. in which the flasks varied from 0.5 to 2.0 L; 1.2-L beakers were used for all other experiments. Each flask or beaker contained filtered water from Heney Lake and a few drops of green algae to serve as food for the prey. The opening of each container (except in Ny 2–Ny 5) was fitted with a hollowed-out rubber stopper containing a piece of 110-μm nylon mesh attached to its bottom (under the water surface) to prevent prey from getting trapped on the surface film. In Ny 2–Ny 5 the beakers were stoppered tightly and rotated on a motorized wheel at about $1\frac{1}{2}$ rpm to ensure an even distribution of predators and prey throughout the experiment. The duration of each experiment was designed to leave more than one-third of the prey alive at the completion.

In series Ad 1, pionid adults were presented with individuals of a single prey species. In Ad 5, size selection by adult mites was tested on various size-classes of *Daphnia pulex* (0.6–2.3 mm). All *Daphnia* (both alive and eaten) were measured from the top margin of the head to the base of the shell spine at the completion of each Ad 5 experiment. Carapaces of individuals eaten remained intact, allowing accurate measurements of body length. The results of the 10 replicates in this series were then combined to determine if any selectivity occurred over this size range in *Daphnia*. All other experiments involved selection by mites from among several

TABLE 1. Summary of design of predation experiments.

Series	No. of trials	Time[a]	Temp.[b]	Predator			Prey			
				Type	No.	Stvn.[c]	Species[d]	No.[e]	Offered[f]	Density[g]
Ad 1	2–11 each	10–24	15	Pionid adults	3–5	0	Bos, Cer Dpu, Dgm Dma, Dus Mes	25	1	50
Ad 2	4	12	15	Pionid adults	3	0	Bos, Cer Dpu, Dma Mes	10	5	42
Ad 3	6	12	15	Pionid adults	4	0	Bos, Cer Chy, Dpu Dma	15	5	62.5
Ad 4	6	18	21	*Piona constricta* (females)	3	24	Dpu, Mes	30	2	50
Ad 5	10	18	15	Pionid adults	2	0	Dpu	60	1[h]	50
Ny 1	4	18–22	21	*P. constricta* (nymphs)	5–6	24	Bos, Cer Chy, Dpu Dma	15	5	62.5
Ny 2	6	20–24	21	*P. constricta* (nymphs)	6	—	Bos, Cer Chy, Dpu Dma	15	5	62.5
Ny 3	6	22–25	21	*P. constricta* (nymphs)	6	24	Bos, Cer Chy, Dpu Dma	15	5	62.5
Ny 4	1	24	21	*P. constricta* (nymphs)	7	51	Bos, Dus	25	2	42
Ny 5	3	24	21	*P. constricta* (nymphs)	6	75–145	Bos, Chy Dus	20	3	50

[a]Duration of experiment (h).
[b]Water temperature (°C).
[c]Period of predator starvation prior to experiment (h).
[d]Bos, *Bosmina*; Cer, *Ceriodaphnia*; Chy, *Chydorus*; Dpu, *Daphnia pulex*; Dgm, *Daphnia galeata mendotae*; Dma, *Diaphanosoma*; Dus, *Diaptomus*; Mes, *Mesocyclops*.
[e]Number of prey of each species offered to predator.
[f]Number of prey species offered to predator at one time.
[g]Total initial prey density for all species combined (no./L).
[h]*Daphnia pulex* of different size-classes (0.6–2.3 mm) offered to pionid adults to test for size selection on this species.

different species of prey which were presented simultaneously. *Piona constricta* nymphs for Ny 1 and Ny 4–Ny 5 were collected from Heney Lake on May 12, 1979 and August 10, 1979, respectively. Nymphs used in Ny 2 and Ny 3 developed from eggs laid by females in glass chambers. Those in Ny 2 were only 1–2 d old and had no previous feeding experience; the same individuals were used in Ny 3, but they had been fed in the interim a diet consisting mostly of *Bosmina* (with some *Ceriodaphnia*). Nymphs used in Ny 1, Ny 3, Ny 4, and Ny 5 were much larger and faster than those in Ny 2.

At the completion of each predation experiment, the zooplankton contents were filtered out and the prey were recorded as alive, dead but not eaten, or eaten. Several replicates, each with its own control (lacking mites), were run for each series of experiments. Mortality in control containers was subtracted from that in the experimental ones to obtain estimates of predation rates on prey species. Control mortality was low, ranging between 0 and 2.5%, except in Ad 4 where it reached 5.8%, primarily because of high mortality in one replicate.

Feeding rates were calculated as both the number of prey eaten per predator per day (predation rate) and the instantaneous feeding rate coefficient K (Dodson 1975):

$$K = \frac{-\ln(P_T/P_I)}{XT}$$

where P_I = initial density of prey (number/L), P_T = density of prey after time T, and X = density of the predator. The dimensions of K are L/d. This latter parameter allows direct comparisons to other studies on invertebrate planktivores (Dodson 1975; O'Brien et al. 1979; O'Brien and Schmidt 1979; Cooper and Goldman 1980). Ivlev's index of electivity, E (Ivlev 1961), was used to compare selection on various prey:

$$E = \frac{r_i - p_i}{r_i + p_i}$$

where r_i = percent composition of prey class i in the diet, and p_i = percent composition of prey class i in the environment. Values of E between 0 and +1 indicate predator preference, values between 0 and −1 predator avoidance, and no selection is indicated if $E = 0$. Student's t-test was used to test for significance in prey selection when two prey species were

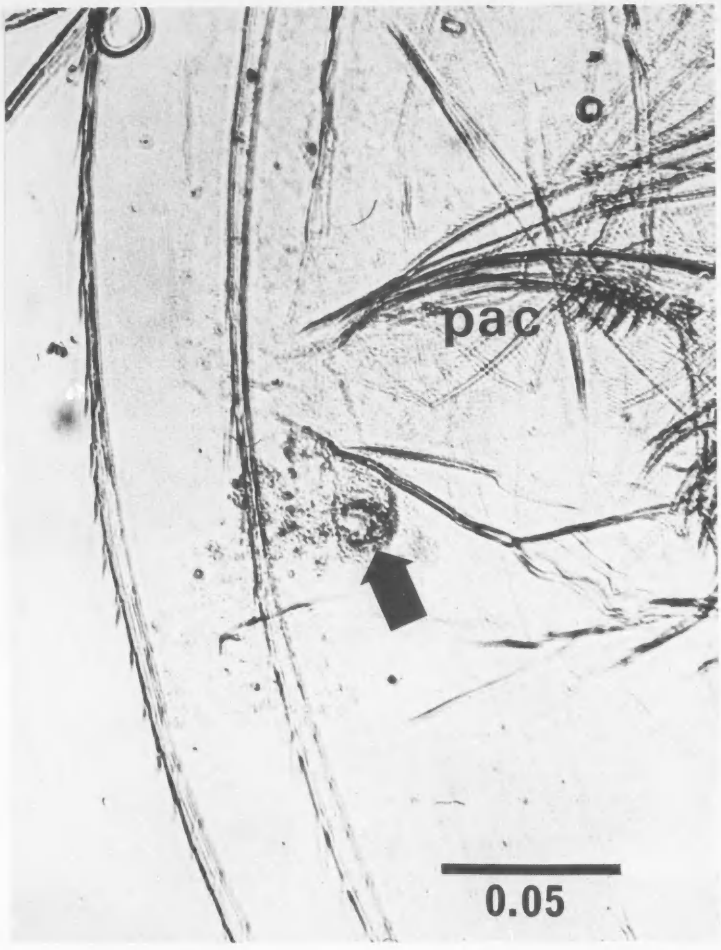

FIG. 1. Portion of ventral, posterior region of carapace of *Daphnia pulex*, showing hole torn in exoskeleton (arrow) by chelicerae of *Piona constricta* adult during predation. Also shown is postabdominal claw (pac). Scale in mm.

used and ANOVA was used similarly for multiprey selection experiments.

Predatory Behavior

ENCOUNTER

The encounter rate of a predator with its prey is a function of the density of prey, the encounter radius of the predator, and the mean velocities of both predator and prey (Gerritsen and Strickler 1977). The encounter radius for *P. constricta* (and the related pionid adults studied) is very small, less than the length of the mite itself, and thus it is difficult to determine whether the mite reacts to the presence of its prey before it actually touches it. The mites swim in an apparently random manner under conditions of uniform, undirected light. Adults swim at mean speeds of 20−25 mm/s, much faster than other limnoplanktonic invertebrate predators (see Gerritsen 1980a; Sebestyén 1960). The swimming speeds of nymphs were not determined, but are considerably slower (especially in the younger nymphs). Mites typically swim for short periods, up to a few seconds, on one course, and then switch direction to another. If the mite runs into a solid object, it abruptly changes its heading. Continuous swimming keeps *Piona* adults from sinking and their fast-cruising speeds increase their encounter rate with both prey and mates. The concurrent

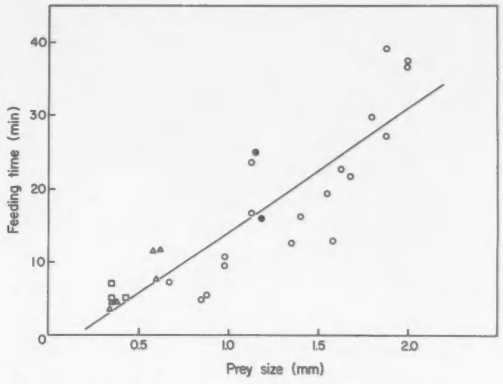

FIG. 2. Relation of feeding time for pionid adults to prey size. Open circles, *Daphnia pulex*; closed circles, *Daphnia galeata mendotae*; triangles, *Ceriodaphnia*; squares, *Bosmina*. Solid line is linear regression on points ($y = -2.67 + 16.84x$, $r = 0.882$).

disadvantage of increasing encounters with potential predators is of minor importance as water mites suffer little from predation (Pieczyński 1976; Riessen 1980, 1981).

Swimming speeds of the various potential prey are usually less than 2 mm/s (Gerritsen 1978, 1980a, b; Swift and Fedorenko 1975; Kerfoot 1978; Strickler 1975b), much slower than those of either the adult or nymphal mites. As the swimming speed of a mite is so much greater than that of its zooplankton prey, the slight differences among the various prey species will have little, if any, effect on their respective encounter rates with the predator (Gerritsen and Strickler 1977). Differing encounter rates for the mite with each potential prey species, therefore, will be due to differences in the population densities of the various prey.

ATTACK AND CAPTURE

When a prey item is encountered, the mite attempts to grab it; using its four pairs of legs and pair of palps, the predator makes a quick, short lunge at its prey from close range. Once the prey has been grabbed, the mite begins to sink through the water, its legs occupied and unable at this moment to function as swimming organs. During this time the mite attempts to secure the prey in a favorable position for feeding, often by rotating it with its legs and palps. When the prey is finally subdued and secured, its exoskeleton is pierced by the chelicerae of the mite (Fig. 1), except when *Chydorus* is the prey. At this point the mite can hold the prey item in position for feeding using only its palps and chelicerae, and the legs are freed for swimming (if the prey is not too large) or anchoring the predator on some suitable substrate (if the prey is too large to swim with). The entire process of attack and capture takes from a couple of seconds for small prey (*Bosmina*, *Ceriodaphnia*) to several seconds for large *Daphnia*, which can put up a considerable struggle and even occasionally escape after being grabbed. When *Chydorus* is the prey, the mite appears to insert its chelicerae between the valves of the cladoceran's carapace — the exoskeleton is apparently too hard to be pierced directly. A somewhat similar process has been reported by Uchida (1932) for predation by *Arrenurus* on ostracods.

INGESTION

In typical arachnid fashion, the water mite predigests its food externally by secreting enzymes from an oral gland into the body of the prey. The digested liquid contents are then drawn into the mouth of the predator by means of a pharyngeal pump (Mitchell 1962; Barnes 1974). When the mite is finished feeding, it drops the dead prey organism and swims away. The length of time the mite spends feeding is directly proportional to the size of the prey item: for pionid adults from Heney Lake, from 3 to 5 min for *Bosmina* and juvenile *Ceriodaphnia* up to 35–40 min for large adult *Daphnia pulex* (Fig. 2). Ellis-Adam and Davids (1970) reported that feeding times for adult *Piona alpicola* (= *P. uncata*) varied from 10 to 50 min on daphnids of unreported size.

The relative destruction of the prey during predation depends on its size. The smaller *Bosmina* and *Ceriodaphnia* show nearly total breakdown of their internal tissues, with the most obvious change being the disintegration of the compound eye. This leaves only the hard chitinous parts, especially the carapace, intact (Fig. 3). This condition is somewhat different than for a *Bosmina* eaten by a copepod, where the eye is often left intact (Kerfoot 1978). In the larger *Daphnia*, dissolution of the body tissues is only partial and some soft tissue remains at the end of predation. The eye, however, nearly always shows some degree of disruption. The carapace itself is undamaged, with the exception of the small hole torn in it by the chelicerae. Digestive enzymes of the mite enter the prey through this hole and must move relatively long distances in the larger prey; some enzymes are undoubtedly lost to the environment in cladoceran prey as a result of the structure of their bivalved carapace. Thus large cladocerans remain only partially digested at the completion of ingestion.

Strangely, eggs in the brood chambers of cladocerans captured and ingested by water mites did not show the tissue breakdown that is characteristic in the parent. Eggs appeared viable and often developed normally to free-swimming juveniles; embryos showing advanced development and capable of some movement were on two occasions seen escaping successfully from the brood chamber of the adult during ingestion. Plankton samples from Heney Lake in June of 1978 (a period of high population densities for both *P. constricta* adults and *Bosmina*) revealed many dead *Bosmina* that had the characteristic appearance of mite predation (see Riessen 1981). Several of these still had intact eggs and embryos in their brood chambers, and in one case the embryo had completely developed into a neonate.

Uchida (1932) described the general process of prey capture and ingestion for *Piona carnea*, and Ellis-Adam and Davids (1970) did the same for *P. uncata*. Their descriptions are remarkably similar to the process observed for *P. constricta* and the other pionid mites in the zooplankton community of Heney Lake.

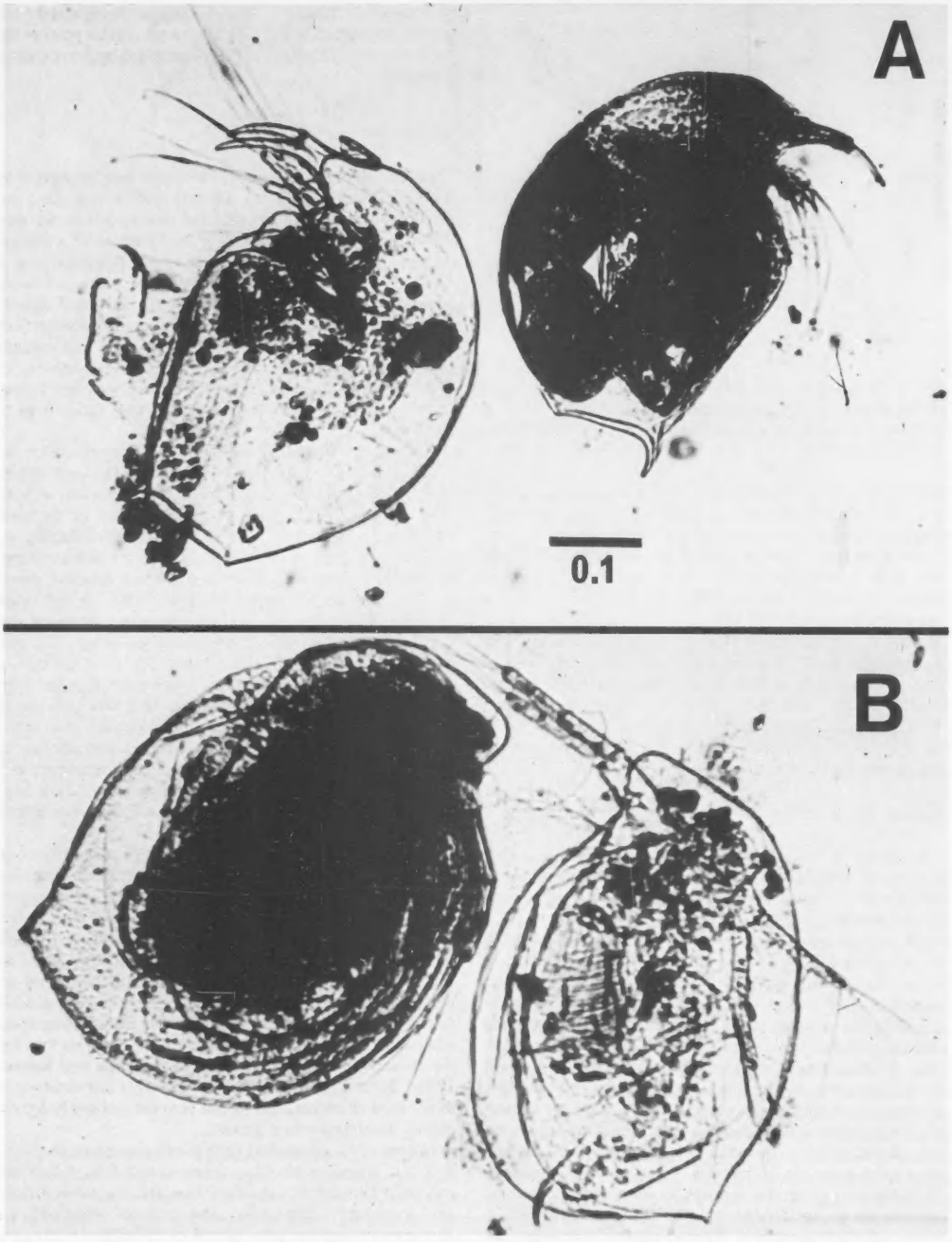

FIG. 3. Prey organisms in normal condition and after attack and ingestion by *Piona constricta* adult. A, *Bosmina longirostris* (normal condition on right). B, *Ceriodaphnia lacustris* (normal condition on left). Scale in mm.

TABLE 2. Predation series Ad 1. Feeding rates of pionid adults on individual prey species, calculated as predation rate (number prey eaten·predator^{-1}·d^{-1}) and instantaneous feeding rate coefficient, K.

Prey type[a]	Mean prey size (mm)	n	Predation rate $\bar{x}$	SE	K
Cladocera					
Bosmina (ad)	0.39	6	10.32	1.33	0.406
Ceriodaphnia (juv)	0.39	6	8.50	1.73	0.263
Ceriodaphnia (ad)	0.61	6	11.13	1.42	0.378
Diaphanosoma (ad)	0.72	7	1.66	0.38	0.037
Daphnia pulex (sm juv)	1.01	11	8.48	0.81	0.288
D. pulex (lg juv)	1.23	5	7.01	0.53	0.181
D. galeata mendotae (juv)	0.96	2	10.67	2.00	0.360
D. galeata mendotae (ad)	1.57	2	8.00	0.35	0.215
Daphnia spp.[b] (juv)	1.05	5	10.80	1.28	0.734
Copepoda					
Mesocyclops (cop)	0.76	6	0.34	0.14	0.008
Mesocyclops (ad)	1.08	6	0.44	0.24	0.010
Diaptomus (cop)	0.86	2	1.42	0.11	0.033
Diaptomus (ad)	1.05	4	1.47	0.10	0.034

[a]ad, adults; juv, juveniles; cop, copepodites.
[b]Composed mostly of *D. pulex* with some *D. galeata mendotae*. Container volumes varied between 0.5 and 2 L for different replicates.

Prey Selectivity

ADULTS

In series Ad 1 (Table 2), where one prey species at a time was offered to pionid adults, predation rates fall into two groups: prey eaten in high numbers (7−11 prey·mite^{-1}·d^{-1}) and prey eaten in low numbers (0−2 prey·mite^{-1}·d^{-1}). The first group (vulnerable prey) is composed of *Bosmina*, *Ceriodaphnia*, and *Daphnia*, whereas the second (resistant prey) includes the copepods and the cladoceran *Diaphanosoma*. All pairwise comparisons of predation rates between members from the resistant prey group and those from the vulnerable prey group showed significant differences (one-way ANOVA, $P < 0.05$), except for the following pairs (in which the number of replicates was small): *Daphnia pulex* (lg juv)−*Diaptomus*, *Daphnia galeata mendotae* (ad)−*Diaptomus*, and *Daphnia galeata mendotae* (ad)−*Diaphanosoma*. Comparisons of predation rates within both the vulnerable and resistant prey groups showed no significant differences between species. Series Ad 1 suggests that *Bosmina*, *Ceriodaphnia*, and *Daphnia* are strongly selected by pionid adults over copepods and *Diaphanosoma*, but true selection experiments, in which several prey species are offered to the predator at once, were needed to confirm this pattern.

Such selection experiments were carried out in series Ad 2, Ad 3, and Ad 4; these both confirmed and expanded upon the pattern established above. In Ad 2 (Fig. 4), the order of prey selection was as follows: *Bosmina* > *Daphnia* > *Ceriodaphnia* > *Diaphanosoma* > *Mesocyclops*. Mean values for predation rate and K on all prey were 11.83 prey·mite^{-1}·d^{-1} and 0.351, respectively. In series Ad 3 (Fig. 4) the order of selection by pionid adults was *Bosmina* > *Ceriodaphnia*, *Daphnia* > *Chydorus* >> *Diaphanosoma*. Mean values for predation rate and K on all prey were 18.83 prey·mite^{-1}·d^{-1} and 0.419, respectively. The results of series Ad 4 (Table 3) show that *Daphnia* is greatly selected over *Mesocyclops* by *P. constricta* females (t-test, $P < 0.001$).

Size selection by pionid adults on *Daphnia pulex* was examined in Ad 5 (Fig. 5). As is typical for invertebrate planktivores (see Dodson 1974b), these mites selected the smaller prey items over larger ones. Electivity values were positive between 0.6 and 1.3 mm prey body length and negative between 1.3 and 2.3 mm. In Heney Lake, adult *Daphnia* only rarely exceed 1.5 mm in length (personal observation), and thus this strong selection pattern is greatly reduced in the natural environment. Mean values for predation rate and K in this set of experiments were 16.16 prey·mite^{-1}·d^{-1} and 0.413, respectively.

Given preferred food items, the adult pionid mites tested in these experiments ate an average of 10−15 prey·mite^{-1}·d^{-1}. The general pattern of prey selection by the mites, including *P. constricta*, is as follows: *Bosmina* > *Daphnia*, *Ceriodaphnia* > *Chydorus* >> *Diaphanosoma*, *Diaptomus* > *Mesocyclops*. Size probably plays some role in this selection by adults, but the results of these experiments cannot be explained solely as a function of size.

NYMPHS

Prey selection by *P. constricta* nymphs on cladocerans from Heney Lake (Ny 1, Ny 2, Ny 3) is shown in Fig. 6. These experiments are the same as, and therefore directly comparable to, series Ad 3 for pionid adults. In Ny 1, where the nymphs were taken directly from Heney Lake on May 12, 1979 (having just completed their winter diapause), the order of prey selection was as follows: *Chydorus* > *Bosmina* > *Ceriodaphnia*, *Daphnia*, *Diaphanosoma*. Total mean predation rate and K were 3.35 prey·mite^{-1}·d^{-1} and 0.057,

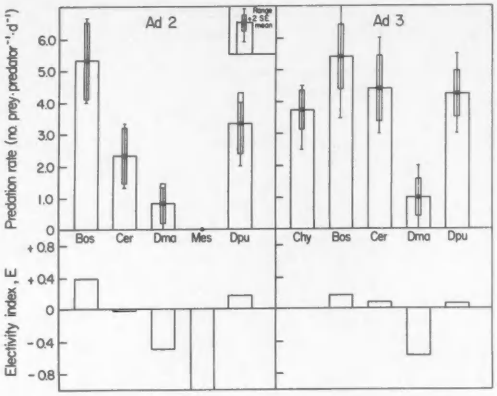

FIG. 4. Predation series Ad 2 and Ad 3. Prey selection by pionid adults on several zooplankton species. Top panels indicate predation rate (mean values ± 2 standard errors and range); bottom panels show Ivlev's index of electivity, *E*. Prey species ordered (from left to right) in increasing size. See Table 1 for key to prey species.

TABLE 3. Predation series Ad 4. Prey selection by *Piona constricta* females on *Daphnia pulex* and *Mesocyclops edax*.

Prey	Mean prey size (mm)	Predation rate $\bar{x}$	SE	*K*
Daphnia pulex[a]	1.16	8.30	1.09	0.590
Mesocyclops edax[b]	0.93	0.07	0.07	0.003

[a]Juveniles.
[b]Large copepodites and adults.

respectively. In Ny 2, the *P. constricta* nymphs, raised in the laboratory from eggs, were newly emerged from the nympho-chrysalis stage (1−2 d) and had no prior feeding experience. Their order of prey selection was *Bosmina* >> *Chydorus*, *Daphnia* > *Ceriodaphnia*, *Diaphanosoma*. Mean values for predation rate and *K* on all prey were 1.89 prey · mite^{-1} · d^{-1} and 0.033, respectively. Finally, in Ny 3 the same nymphs used in Ny 2 were retested after having grown larger on a diet consisting primarily of *Bosmina* (with some *Ceriodaphnia*). Their prey selection after feeding was essentially the same: *Bosmina* >> *Chydorus* > *Daphnia* > *Ceriodaphnia*, *Diaphanosoma*, except that *Chydorus* was eaten in higher percentages than before. Total mean predation rate and *K* were 2.40 prey · mite^{-1} · d^{-1} and 0.043, respectively.

Series Ny 4 and Ny 5 tested *P. constricta* nymphs collected directly from Heney Lake on August 10, 1979, and examined prey preference on the cladocerans *Bosmina* and *Chydorus* and the copepod *Diaptomus*. In Ny 4 (Table 4), there is a clear selection for *Bosmina* over *Diaptomus*; indeed there is no predation at all on the copepod in this experiment. In Ny 5 (Fig. 7), the nymphs ate an average of 2.11 prey · mite^{-1} · d^{-1} (mean value of $K = 0.047$), and the order of prey selection was *Bosmina*, *Chydorus* >> *Diaptomus*.

The average predation rate of *P. constricta* nymphs is 2−3 prey · mite^{-1} · d^{-1}, about one-fifth that of adults.

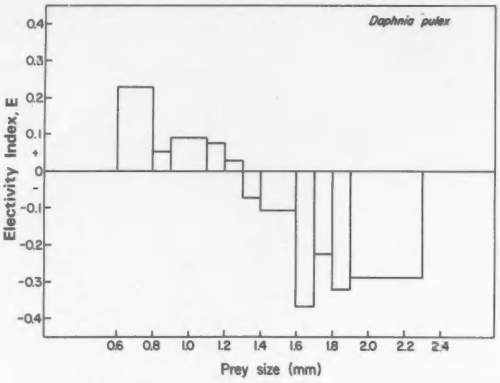

FIG. 5. Predation series Ad 5. Size-selective predation by pionid adults on different size-classes of *Daphnia pulex*. Selection indicated by Ivlev's index of electivity, *E*.

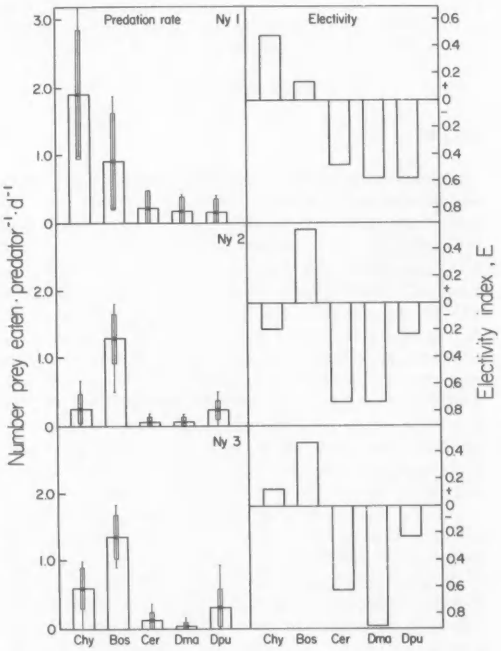

FIG. 6. Predation series Ny 1, Ny 2, and Ny 3. Prey selection by *Piona constricta* nymphs on several zooplankton species. Panels on left indicate predation rate (mean values ± 2 standard errors and range, see Fig. 4); panels on right show Ivlev's index of electivity, *E*. Prey species ordered (from left to right) in increasing size. See Table 1 for key to prey species.

Bosmina and *Chydorus* are the preferred prey of the nymphs, while the other cladocerans (*Ceriodaphnia*, *Diaphanosoma*, and *Daphnia*) and the copepods (*Diaptomus*) are only rarely

TABLE 4. Predation series Ny 4. Prey selection by *Piona constricta* nymphs on *Bosmina longirostris* and *Diaptomus* sp.

Prey	Mean prey size (mm)	Number of *Piona* nymphs	Number of prey		Time (h)	Predation rate	*K*
			Initially	Eaten			
Bosmina longirostris	0.37	7	25	17	24	2.43	0.195
Diaptomus sp.	0.87		25	0		0	0

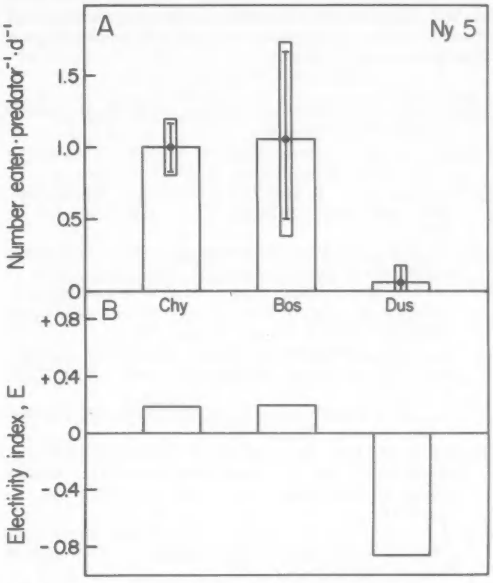

FIG. 7. Predation series Ny 5. Prey selection by *Piona constricta* nymphs on *Chydorus* (Chy), *Bosmina* (Bos), and *Diaptomus* (Dus). A, predation rate (mean values ± 2 standard errors and range, see Fig. 4). B, Ivlev's index of electivity, *E*. Prey species ordered (from left to right) in increasing size.

eaten. In general, prey selection by nymphs is similar to that of adults; however, the nymphs have a more restricted diet — *Daphnia* and *Ceriodaphnia* are in the group of prey preferred by adults but are not eaten in great numbers by nymphs.

The relative predation by *P. constricta* nymphs on *Bosmina* and *Chydorus* varies considerably in these experiments and appears to depend on the age and prior feeding experience of the predator. Nymphs newly emerged from the nymphochrysalis stage and without prior feeding experience (Ny 2) showed a strong preference for *Bosmina* over *Chydorus*. This preference is still present, but to a lesser degree, when these nymphs are older and larger, having been fed *Bosmina* in the interim (Ny 3). Nymphs taken from Heney Lake in August (Ny 5) prey on both species in equal proportions — these nymphs had been exposed to the first part of the regular summer "bloom" of *Chydorus* in the plankton of Heney Lake (see Riessen 1981). Nymphs taken from the lake in May (Ny 1) strongly selected *Chydorus* over *Bosmina* — these nymphs had been exposed the previous year to the complete *Chydorus* "bloom" (see Riessen 1981). *Bosmina* densities are very low in Heney Lake during the period when the nymphs are active (mid-July–September) (Riessen 1981). The strong preference of young, unfed nymphs for *Bosmina* lessens as the nymphs grow older and gain feeding experience, even if this experience is with *Bosmina*. If the feeding experience is with *Chydorus*, this initial preference for *Bosmina* will disappear, resulting in either no preference (if the exposure to *Chydorus* is relatively short as in Ny 5) or preference for *Chydorus* (if the exposure to *Chydorus* is relatively long as in Ny 1).

Discussion

The few other studies which have investigated predation by water mites on zooplankton are generally in accord with this one. Gliwicz and Biesiadka (1975) found that *P. limnetica* showed a distinct preference for cladocerans over both rotifers and copepods (cyclopoids and calanoids). They did find, however, that *Diaphanosoma* was among the preferred prey, in addition to *Bosmina* and *Ceriodaphnia*. They estimated the predation rate of the water mites at between 10–20 cladocerans·mite^{-1}·d^{-1}, very close to the values determined above for pionid adults. Paveljeva and Zánkai (1971) tested the prey selection of a variety of water mites on five cladoceran species. They found that *Piona coccinea* greatly preferred *Bosmina* to *Daphnia*, which in turn was followed, in order of selection, by *Ceriodaphnia*, *Simocephalus*, and *Sida*. The general pattern for all mites tested showed both *Bosmina* and *Daphnia* as the most preferred prey. Ellis-Adam and Davids (1970) reported that adult *P. uncata* preferred daphnids to copepods and that the predation rate could reach 10 prey·mite^{-1}·d^{-1}.

The different morphologies and behaviors of the various prey species within the crustacean zooplankton community make some more vulnerable than others to predation by water mites. *Bosmina* and *Chydorus* are relatively small and do not have an accelerated escape reaction to evade an approaching mite. They execute instead a "dead-man response" (Kerfoot 1977, 1978) and sink passively through the water. *Daphnia* and *Ceriodaphnia* are considerably larger and both show a definite escape behavior — these prey often, but not always, make short jumps away from an approaching predator before they are physically contacted (see Szlauer 1964). This escape reaction is limited, however, in both speed and distance travelled; Cushing (1955) estimated the maximum speed of *Daphnia longispina* at about 6 cm/s. *Diaphanosoma* and the

copepods (*Diaptomus* and *Mesocyclops*), also relatively large, show fast, efficient, escape reactions to approaching objects. Swift and Fedorenko (1975) reported that, if disturbed, *Diaptomus kenai* can travel 3–10 cm in a single jump at a mean speed of 147 cm/s, while *Diaphanosoma* can leap 3–6 cm at a time. *Cyclops scutifer* can execute an escape response at speeds as high as 50 cm/s (Strickler 1975a).

Neither *Diaphanosoma* nor copepods are preyed upon to any great degree by water mites (nymphs or adults), and this appears to be a function of their efficient escape behavior. When confined in a small volume (1 mL) of water with a mite, *Mesocyclops* are occasionally captured, and although they struggle for a couple of seconds, they never manage to escape. The handling and ingesting of these prey appear to present no problem to the mites. This escape behavior significantly lowers the probability of copepods being captured by planktivorous fish (Drenner et al. 1978) and under normal conditions appears to have the same effect on water mites.

Both *Bosmina* and *Chydorus* are heavily preyed upon by the water mites. Their small size and the lack of an accelerated escape reaction leaves them extremely vulnerable to both the nymphs and adults. Once a *Bosmina* is captured, it is invariably ingested; thus the morphological features of this zooplankter that are so effective against copepod predation (Kerfoot 1975, 1977, 1978) are useless against water mites. Although *Chydorus* are also easily captured, their relatively thick carapace may offer some protection in preventing ingestion. On one occasion an adult pionid was observed attempting to eat a *Chydorus*: the prey was captured without a struggle and the mite spent more than 2 min manipulating it, the entire process culminating in the release of the chydorid, which sank to the bottom of the dish and then swam away seemingly unharmed. This "armor" probably explains the preference of both the adult mites and the younger nymphs for *Bosmina* over *Chydorus*. As the nymphs obtain experience with *Chydorus* (apparently becoming more efficient at handling these prey), this preference disappears.

Daphnia (0.8–1.0 mm) and *Ceriodaphnia* are preyed upon heavily by pionid adults, but only very lightly by the nymphs. These prey are probably too large or too quick to be successfully preyed upon by the nymphs, while neither large enough nor quick enough to prevent predation from adults. Once initially seized by adult mites, *Ceriodaphnia* are always successfully ingested. Smaller *Daphnia* (< 1.5 mm) are infrequently able to free themselves after being contacted by a mite, while larger *Daphnia* (> 1.5 mm) often escape death by thrashing about with their antennae and pulling free before the mite has manipulated them into position for the chelicerae to act. This differential ability to escape after being initially grabbed by the predator is the probable reason for the selection of smaller *Daphnia* by adult mites. For the smaller nymphs (about half the length of the adults), prey in the size range 0.5–1.0 mm may be too large to handle efficiently, making predation on *Daphnia* and *Ceriodaphnia* difficult.

Gliwicz and Biesiadka (1975) have shown that *P. limnetica* does not prey on rotifers. This is probably a function of the rotifers being too small to be either recognized as prey, manipulated easily, or successfully ingested.

The pattern of prey selection by both adult mites and nymphs appears to depend primarily on the ability of prey to detect an approaching mite and flee the area. Prey size and the thick carapace of *Chydorus* are of secondary importance, and the presence of spines in *Bosmina* and *Daphnia* appears not to play a role.

Acknowledgments

I am deeply grateful to J. R. Strickler for his aid and for the intellectual climate he provided. I also thank D. Smith, D. Safar-Riessen, I. M. Smith, and C. K. Wong for their help and advice, the Department of Biology at the University of Ottawa for its hospitality, and W. J. O'Brien and W. M. Lewis Jr. for reviewing the manuscript. Financial support was provided by National Research Council of Canada grants to J. R. Strickler.

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Susceptibility of Trouts of Different Species and Origins to Various Isolates of Infectious Pancreatic Necrosis Virus

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SILIM, A., M. A. S. Y. ELAZHARY, AND A. LAGACÉ. 1982. Susceptibility of trouts of different species and origins to various isolates of infectious pancreatic necrosis virus. Can. J. Fish. Aquat. Sci. 39: 1580 – 1584.

We investigated the susceptibility of trouts of different species and origins to infectious pancreatic necrosis virus (IPNV) and the pathogenicity of three strains of IPNV for brook trout (*Salvelinus fontinalis*) of various origins and lake trout (*Salvelinus namaycush*) of a single origin. Fish were inoculated by immersion in water containing 10^5 PFU of virus/mL for 6 h. Susceptibility to IPNV infection was assessed by counting dead fish over a period of 21 d after infection and on histological lesions in the pancreas, kidney, and intestine of the infected fish. Different species of trouts had different susceptibility to IPNV strain 3865. Brook trout had the highest mortality followed by rainbow trout (*Salmo gairdneri*), whereas the least mortality occurred in lake trout. Brook trout from Crowford, Nebraska, were more than twice as susceptible as the same species from Baldwin Mills, Quebec. Rainbow trout also varied in susceptibility as a result of origin. Virus isolate 3B, originally isolated from chain pickerel (*Esox niger*), was less virulent than isolates 4495 or 3865 (both from trout). Hatcheries that use water from sources containing pickerel may increase their chances of IPN infection.

Key words: trout species, infectious pancreatic necrosis virus, resistance

SILIM, A., M. A. S. Y. ELAZHARY, AND A. LAGACÉ. 1982. Susceptibility of trouts of different species and origins to various isolates of infectious pancreatic necrosis virus. Can. J. Fish. Aquat. Sci. 39: 1580 – 1584.

Nous avons étudié la prédisposition de truites de différentes espèces et origines au virus de la nécrose pancréatique infectieuse (IPNV) et la virulence de trois lignées de ce virus chez des ombles de fontaine (*Salvelinus fontinalis*) d'origines diverses et des touladis (*Salvelinus namaycush*) de même origine. Les poissons ont été inoculés par immersion dans de l'eau contenant 10^5 PFU de virus/mL pendant 6 h. La prédisposition à l'infection a été évaluée par comptage des poissons morts pendant une période de 21 jours après infection et par examen histologique des lésions dans le pancréas, le rein et l'intestin des poissons infectés. La prédisposition à la lignée 3865 de l'IPNV varie selon l'espèce de truite. L'omble de fontaine a les mortalités les plus élevées. Vient ensuite la truite arc-en-ciel (*Salmo gairdneri*), alors que le touladi est l'espèce la moins sensible. L'omble de fontaine provenant de Crowford, au Nebraska, est plus de deux fois plus sensible que la même espèce provenant de Baldwin Mills, au Québec. La prédisposition de la truite arc-en-ciel varie également selon l'origine. L'isolat 3B du virus, provenant du brochet maillé (*Esox niger*) est moins virulent que les isolats 4495 ou 3865 (provenant tous deux de la truite). Les écloseries qui utilisent de l'eau contenant des brochets risquent donc une infection par l'IPNV.

Received January 29, 1982
Accepted August 23, 1982

Reçu le 29 janvier 1982
Accepté le 23 août 1982

INFECTIOUS pancreatic necrosis is a viral disease of trout fry of widespread distribution throughout Canada (Elazhary et al. 1976; MacKelvie and Artsob 1969; Sonstegard and McDermott 1971; Yamamoto 1974) and the United States (Parisot et al. 1963, 1965). Mortalities of 62–100% have been reported by Snieszko et al. (1957, 1959) and Wood et al. (1955). In Denmark, mortalities of 5–90% have been reported by Vestergard-Jorgensen and Kehlet (1971). There is inadequate information explaining the great variation in mortality rates. Some authors (Wood et al. 1955; Wolf and

Printed in Canada (J6799)
Imprimé au Canada (J6799)

Quimby 1969; Snieszko et al. 1959; Frantsi and Savan 1971) have suggested that mortality is inversely proportional to age of the fish. Frantsi and Savan (1971) also found a linear association between water temperature and mortality.

In an outbreak of IPN infection in trout in a hatchery, Elazhary et al. (1976) noted that the origin and species of trout might have influenced the resistance of the fish to the virus. Reports on genetic differences between origins or species of fish to resistance to viral infections are scarce. This study was undertaken to investigate the influence of species and origins of fingerling trout on their resistance to various isolates of infectious pancreatic necrosis virus (IPNV).

Materials and Methods

Experimental Fish

Three species of trouts from seven different sources with no known history of IPN were used: rainbow trout (*Salmo gairdneri*) (RBD 80-74 and RBD 80-77) from Baldwin Mills, Quebec; brook trout (*Salvelinus fontinalis*) (BBM) from Baldwin Mills; brook trout (BGP) from Gaspé, Quebec; brook trout (BNB) from Crowford, Nebraska; lake trout (*Salvelinus namaycush*) (L30000) from Lake 30000, Quebec; lake trout (LLM) from Lake Massawapi, Quebec. The fish were hatched in a hatchery in Baldwin Mills, maintained at 8–9°C, and transferred to the experimental facility at 4–5 wk of age. They were immediately divided into groups of 350 individuals and held at a water temperature of 9–10°C for 7 d before use in the experiment. During this time the fish were observed daily and dead fish from each group were submitted for viral isolation in cell cultures. The trout were inoculated at 5–6 wk of age.

Viral Source

Three different isolates of IPNV isolated in 1978 were used in this study. Isolate 4495 was from brook trout in which it caused 5% mortality. Isolate 3865 was from brook trout with 51% mortality. Isolate 3B was from chain pickerel (*Esox niger*) from Cabonga Reservoir in Parc La Vérendrye, Quebec.

All isolates were identified as IPN virus by electron microscopy and by serum neutralization test using antisera to IPNV strains VR-299, Ab, and SP (supplied by Dr M. Dorson, Laboratoire d'ichtyopathologie, 78850 Thiverval, Grignon, France) as described by Elazhary et al. (1976). The isolates had the highest serum-neutralizing index to serotype VR-299 and were considered as belonging to this serotype.

Cell Culture Method

Rainbow trout gonad (RTG-2) cell cultures were grown in 24-well Linbro plastic tissue culture plates or in P16 Linbro plastic tissue culture flasks (Flow Laboratories, Hamden, CT). Growth medium consisted of MacPherson and Stocker's modified Eagle's minimum essential medium (MEM) (Eurobio, Paris, France) supplemented with 10% tryptose phosphate broth (Difco, Detroit, MI), 10% fetal bovine serum (FBS), and 100 IU penicillin/mL and 100 μg streptomycin/mL (Flow Laboratories). They were incubated at 18°C in a 5% CO_2 humid atmosphere. Maintenance medium was the same, except that it contained 2% FBS.

TABLE 1. Mean titers (log_{10} TCID50/g) of virus isolated from different groups of trout fry infected with IPNV isolate 3865.

	Virus titer for each group[a]						
PID	A	B	C	D	E	F	G
3	–	–	+	+	+	–	+
7	2.3	2	5.1	5.3	5.0	4.3	4.5
10	3.3	2	5.3	5.3	5.5	4.3	3.7
12	3.5	2.8	5.3	5.8	5.0	5.3	n.t.
14	3.3	2.3	5.3	5.8	6.0	n.t.	5.7
16	2.3	2.5	4.8	5.3	6.5	5.3	3.7
21	n.t.	2.3	5.3	6.3	n.t.	5.3	n.t.

[a] +, IPNV isolated at first or second passage; –, IPNV not isolated; n.t., not tested.

TABLE 2. Mean titers (log_{10} TCID50) of virus from different groups of trout fry infected with different isolates of IPNV.

	Virus isolate and titer for each group[a]		
PID	3865	3B	4495
	Group C	*Group C_1*	*Group C_2*
3	+	–	–
7	5.1	5.3	4.6
10	5.3	5.1	4.6
12	5.3	5.1	5.6
14	5.3	5.6	5.8
16	4.8	5.3	5.5
21	5.3	6.6	6.1
	Group D	*Group D_1*	*Group D_2*
3	+	–	–
7	+	4.3	5.3
10	5.3	4.8	5.8
12	5.1	5.6	5.8
14	6.1	5.8	5.8
16	5.3	5.3	5.3
21	6.3	6.1	6.3
	Group F	*Group F_1*	*Group F_2*
3	–	+	–
7	4.3	+	5.3
10	4.3	+	4.6
12	5.3	+	5.3
14	n.t.	n.t.	n.t.
16	5.3	+	n.t.
21	5.3	+	5.3

[a] +, virus isolated at first or second passage; –, virus not isolated; n.t., not tested.

Viral Propagation and Titration

The stock virus for infection of fish was prepared by inoculating confluent monolayer cultures of RTG-2 cells in P16

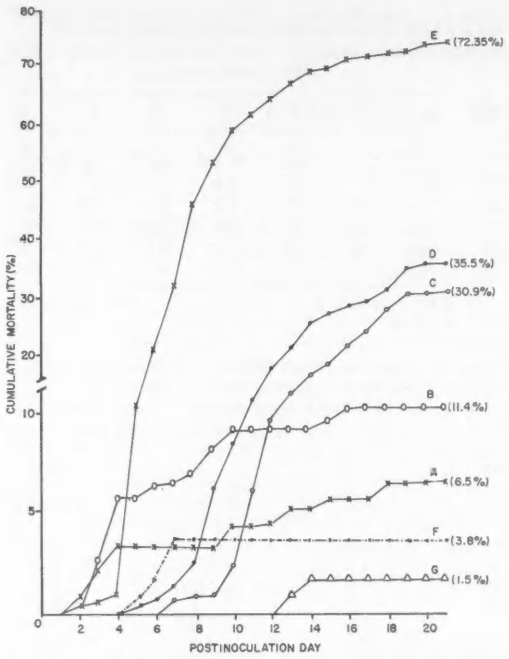

FIG. 1. Cumulative mortalities of different groups of trout infected with IPNV isolate 3865.

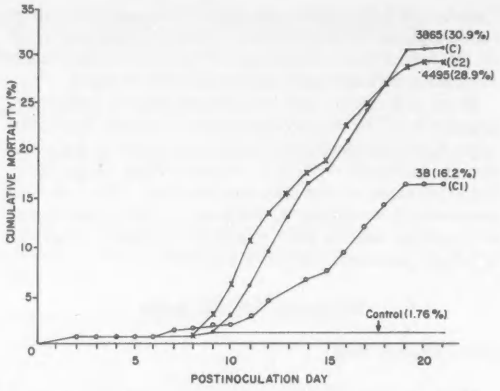

FIG. 2. Cumulative mortalities of brook trout (BBM) from Baldwin Mills infected with the three different IPNV isolates.

flasks. Each flask was inoculated with 4 mL of 1:10 dilution of the original virus containing homogenate and incubated at 19°C for 1 h. The inoculum was removed, the monolayer was washed twice with phosphate-buffered saline (PBS), pH 7.2, and 30 mL of maintenance medium was added into each flask. Control cultures were treated similarly but without virus-containing inoculum. After 4 d of incubation, the virus was harvested and stored at −80°C in 10-mL quantities pending use.

The virus was titrated in 24-well Linbro tissue culture plates by the plaque method of Dobos (1976) using gum tragacanth overlay. Titers were calculated as viral plaque-forming units (PFU)/mL.

FISH INOCULATION PROCEDURE

The various groups of fish were inoculated by immersion in water containing 10^5 PFU of virus/mL for 6 h. Controls were treated similarly with cell culture medium instead of virus. Daily mortality was scored up to postinfection day (PID) 21, and the results were analyzed statistically using the Chi-square test. Dead fish were fixed in formalin for histopathological examination. In addition, three fish were collected from each group at PID 0, 3, 7, 9, 10, 12, 14, 16, and 21 for virus titration. Specimens were stored at −80°C for 2−4 wk pending titration.

EXPERIMENT 1

The aim of this experiment was to investigate the susceptibility of different species and origins of trouts to a single isolate (3865) of IPN virus. Susceptibility was assessed by counting dead fish over a period of 21 d after infection. The fish were divided into the following seven groups:

Group A, rainbow trout RBD 80-74, 307 individuals
Group B, rainbow trout RBD 80-77, 307 individuals
Group C, brook trout, BBM, 310 individuals
Group D, brook trout, BGP, 309 individuals
Group E, brook trout, BNB, 304 individuals
Group F, lake trout, LLM, 316 individuals
Group G, lake trout, L30000, 330 individuals

Seven uninfected groups were manipulated similarly for the controls.

EXPERIMENT 2

The purpose of this experiment was to investigate the susceptibility of brook trout of various origins and lake trout of a single origin to three strains of IPN virus using the same criteria as in experiment 1. The fish were divided into nine groups:

Group C, brook trout, BBM (as exp. 1)
Group C_1, BBM infected with isolate 3B, 308 individuals
Group C_2, BBM infected with isolate 4495, 307 individuals
Group D, brook trout, BGP (as exp. 1)
Group D_1, BGP infected with isolate 3B, 304 individuals
Group D_2, BGP infected with isolate 4495, 307 individuals
Group F, lake trout, LLM (as exp. 1)
Group F_1, LLM infected with isolate 3B, 316 individuals
Group F_2, LLM infected with isolate 4495, 310 individuals

The fish were infected as described and the controls were the same as in groups C to F in experiment 1.

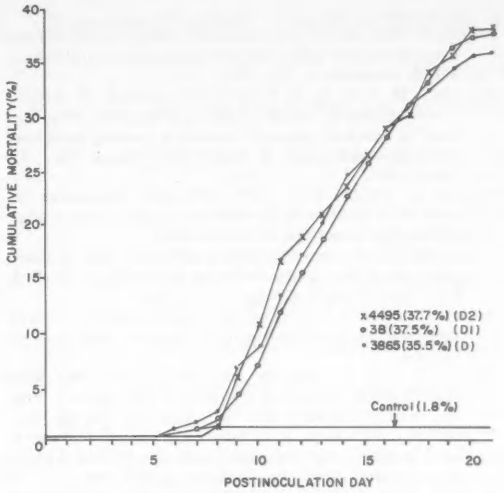

FIG. 3. Cumulative mortalities of brook trout (BGP) from Gaspé infected with the three different IPNV isolates.

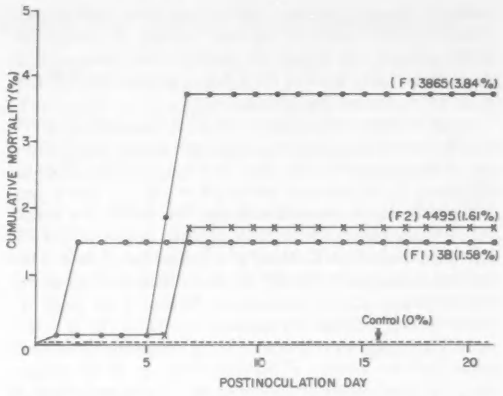

FIG. 4. Cumulative mortalities of lake trout (LLM) from Massawapi infected with the three different IPNV isolates.

VIRAL TITRATION FROM EXPERIMENTAL FISH

The specimens from each day and group were ground separately and diluted 1:10 w/v in maintenance medium. The preparations were centrifuged at 10 000 × *g* for 10 min and the supernatants were filtered through a 0.45-μm filter (Millipore Corporation, Bedford, MA). Tenfold serial dilutions of the filtered extracts were made in MEM and inoculated onto RTG-2 cell cultures grown in 24-well Linbro tissue culture plates, using 0.2 mL/well, three wells/dilution.

After adsorption for 1 h at 19°C, maintenance medium was added and the cultures were incubated for 7 d and examined for cytopathic effect (CPE). Viral titer was calculated according to the Kaerber method (Bross 1950) and was expressed as median tissue culture infective dose (TCID50) per gram.

HISTOLOGICAL EXAMINATION

Dead fish were fixed in 10% formalin and thin sections were cut, stained with hematoxylin and eosin (H&E), and examined with the microscope.

Results

Microscopic lesions of IPN as described by Wolf and Quimby (1971) were observed in the pancreas, kidney, and intestine of all infected trout except lake trout from both Lake 30000 (group G) and Lake Massawapi (group F). Most brook trout from Nebraska infected with isolate 3865 (group E) had diffuse lesions, especially in the pancreas, whereas in other brook trout the lesions were localized or focalized. Lesions were first observed on PID 5 in brook trout from Nebraska, and on PID 10 in brook trout from Gaspé and Baldwin Mills and in rainbow trout. IPN lesions were not observed in the controls.

IPNV was isolated from 5 out of 13 infected groups starting from PID 3 and from all of the infected groups from PID 7 till PID 21 (Tables 1 and 2). The virus was not isolated from control groups.

EXPERIMENT 1

The susceptibility of the seven different trouts to IPNV isolate 3865 differed significantly (Chi-square test, $P < 0.0001$). Highest mortalities (mean 46.25%) were observed in brook trout in group E (Nebraska), group D (Gaspé), and group C (Baldwin Mills) (Fig. 1). Rainbow trout in groups A and B (both from Baldwin Mills) had medium to low mortalities (mean 9%). The lowest mortalities (mean 2.7%) occurred in lake trout in group F (LLM) and group G (L30000). Mortality in control groups ranged from 0 to 1.8%.

EXPERIMENT 2

The virulence of IPNV isolate 3B in trout, disregarding trout species or origin, differed significantly ($P = 0.05$) from the virulence of isolate 3865 or 4495. IPNV isolate 3B caused a total mortality of 18.4% in all the infected trout, and isolates 4495 and 3865 caused 22.7 and 23.4% mortality, respectively. The virulence of the three isolates to brook trout (BBM) from Baldwin Mills was significantly different ($P = 0.0002$); isolate 3B was less virulent than 4495 or 3865 (Fig. 2). On the other hand, no significant difference in virulence was observed among brook trout (BGP) from Gaspé (Fig. 3). Lake trout was the most resistant of all the three trout species, with mortalities of only 1.6 to 3.8% (Fig. 4).

Discussion

Our results demonstrate that various species of trout have different susceptibility to IPNV as indicated by mortality rate following infection with viral strain 3865. Brook trout had the highest mortality, followed by rainbow trout, while the least susceptible was lake trout. A similar study using different

strains of channel catfish (*Ictalurus punctatus*) infected with channel catfish virus and sockeye salmon (*Oncorhynchus nerka*) infected with hemopoetic necrosis virus showed a wide range of mortality between the different strains of fish (Plumb et al. 1975; Amend and Nelson 1977).

Trout fry from some origins were more susceptible to IPNV than those from other origins. Thus, fish origins might play a role in the resistance to the virus. It is not certain whether the difference in the resistance attributed to origin is due to genetic constitution or maternal antibody. The presence of maternal antibody and its role in the resistance of fish to IPNV is not well documented. Elazhary et al. (unpublished data) could not detect maternal antibody by serum neutralization or immunodiffusion test in homogenate filtrate from trout fry whose parents had high seroneutralizing antibody titers to IPN virus. This might have been due to the sensitivity of the tests rather than the absence of maternal antibody. In the present study maternal antibody was not tested for and the hatcheries have not reported IPN infection for the last 5 yr. It was therefore assumed that there were no maternal antibodies as the parent fish were certified IPN free for the stated period of time.

Analysis of the three viral isolates indicated that isolate 3B was less virulent than isolates 4495 and 3865, although its titer appeared to be equal to titers of the other two isolates except for group F_1. The virus was isolated from chain pickerel, and its role in disease in the pickerel is not known. Ahne (1977) infected northern pike (*Esox lucius*) and trout with IPNV isolated from pike fry which was serologically related to strains Sp and Ab. The virus was non-pathogenic for fry of rainbow trout, brook trout, and pike. On the contrary, our viral isolate 3B from pickerel was moderately pathogenic for brook trout and less so for lake trout.

Because some hatcheries use water from sources containing pickerel, it is probable that pickerel could act as reservoirs or carriers for the IPNV which infect the hatcheries.

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Ingestion-Limited Growth of Aquatic Animals: The Case for Blackman Kinetics

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CONDREY, R. E. 1982. Ingestion-limited growth of aquatic animals: the case for Blackman kinetics. Can. J. Fish. Aquat. Sci. 39: 1585–1595.

The published data on ingestion-limited growth in aquatic animals is shown to be consistent with Blackman kinetics. As such, if ingestion limits growth rate there is a linear relationship between growth and ingestion from starvation to satiation. If, however, growth is limited by some factor other than ingestion rate, there is no increase in growth rate with increasing ingestion rate.

Key words: ingestion, growth, growth efficiency, Blackman kinetics

CONDREY, R. E. 1982. Ingestion-limited growth of aquatic animals: the case for Blackman kinetics. Can. J. Fish. Aquat. Sci. 39: 1585–1595.

Nous démontrons ici que les données publiées sur la croissance limitée par l'ingestion chez les animaux aquatiques sont en accord avec la cinétique de Blackman. C'est dire que, si l'ingestion limite le taux de croissance, il y a une relation linéaire entre la croissance et l'ingestion depuis le manque de nourriture jusqu'à la satiété. Si, par ailleurs, la croissance est limitée par un facteur autre que le taux d'ingestion, il n'y a pas d'augmentation du taux de croissance avec une augmentation du taux d'ingestion.

Received June 19, 1981
Accepted August 19, 1982

Reçu le 19 juin 1981
Accepté le 19 août 1982

BLACKMAN (1905) proposed that when a biological process is dependent upon the concentration of several factors, that the rate of the process will be limited by the concentration of a single factor only so long as it is suboptimal in relationship to the other factors. As an example, Blackman demonstrated how the rate of carbon assimilation by algae might be saturated at a given $[CO_2]$ because light intensity had become limiting. When the light intensity was increased, a new plateau was reached in the relationship between $[CO_2]$ and the rate of CO_2 assimilation. Below this plateau CO_2 assimilation was linearly related to $[CO_2]$.

I have shown that Monod's (1942) data on substrate-limited growth of bacteria are consistent with Blackman kinetics, not Michaelis–Menten kinetics (Condrey 1981). In this paper I test whether Blackman kinetics adequately describes the published data I have found on growth and ingestion for aquatic animals. The equation I use is a special case of a piecewise regression of the form

$$(1)\quad G = -M + A \cdot I - A \cdot X \cdot (I - I_x)$$

where

$$X = \begin{cases} 0 \text{ for } I \leqq I_x \\ 1 \text{ for } I > I_x, \end{cases}$$

$I_x = (M + G_x)/A$; G and G_x are the observed and maximum growth rates; M is the maintenance metabolism rate; A is the assimilation = incorporation to ingestion ratio; and I is the ingestion rate (e.g. Condrey 1982; Snedecor and Cochran 1967).

I will first examine the data used to develop more complex models; then review the work of authors who have independently applied Blackman kinetics to their data on growth and ingestion; and, finally, present an examination of other data that I have found.

Materials and Methods

In testing the fit of Blackman kinetics, I fitted a quadratic regression to test for departure from linearity (Snedecor and Cochran 1967). If the quadratic term was not significant, I fitted a linear regression.

If the quadratic term was significant, I fitted Blackman kinetics as expressed by the piecewise regression, Eq. 1. If no other model had been proposed to describe data for which the quadratic term was significant, I also fitted Monod kinetics as it is widely used to describe saturation phenomena. In testing the fit of these models I compared the residual sum of squares as well as the double- and single-reciprocal plots of the data and the predicted relationships for consistency with Blackman or Monod kinetics (e.g. Lineweaver and Burk 1934; Condrey 1981). The latter is a more restricting test than fitting Blackman kinetics to the transformed data, as the equations fitted to the untransformed data do not necessarily track the transformed data well (e.g. Dowd and Riggs 1965).

If the data contained class variables (e.g. sex, species) I tested the quadratic term with each group. If the quadratic term was significant and of the same sign in 25% or less of the

Printed in Canada (J6547)
Imprimé au Canada (J6547)

TABLE 1. Testing the fit of Blackman kinetics to published data on ingestion-limited growth in aquatic animals. Where I plot an author's data in this text, I use the same units in this table as I did in that figure. Where I do not plot an author's data, I use the same units in this table as the author used in his paper. Data sets appear in this table in the same order as their appearance in the text.

	Test of quadratic term			Regression predicted[a]				
Author (case)	*t* statistic	df MSE	*P*	Equation	*F* statistic	df MSE	*P*	r^2
Dawes (1931a, b)								
Males: Cawsand, 1929	−1.70	63	0.09	$G = -0.00 + 0.14I$	87	64	<0.01	0.58
Lympstone, 1929	2.79	71	<0.01	$G = 0.00 + 0.15I$	78	72	<0.01	0.52
Cawsand, 1930	−6.07	167	<0.01	$G = 0.00 + 0.07I$	88	168	<0.01	0.34
Lympstone, 1930	0.60	135	0.55	$G = -0.00 + 0.10I$	91	136	<0.01	0.40
Females: Cawsand, 1929	1.42	85	0.16	$G = -0.00 + 0.16I$	112	86	<0.01	0.56
Lympstone, 1929	0.15	81	0.88	$G = -0.00 + 0.14I$	306	82	<0.01	0.79
Cawsand, 1930	1.70	156	0.09	$G = -0.00 + 0.19I$	306	157	<0.01	0.66
Lympstone, 1930	2.04	122	0.04	$G = -0.00 + 0.11I$	56	123	<0.01	0.31
Gerking (1955)								
summer growth (dry wt)	−1.81	7	0.11	$G = 0.00 + 0.39I$	31	8	<0.01	0.80
fall growth (dry wt)	−0.34	7	0.74	$G = -0.01 + 0.64I$	65	8	<0.01	0.89
Mullin and Brooks (1970)								
Gymnodinium	−1.76	9	0.11	$G = 0.27 + 0.21I$	21	10	<0.01	0.68
Thallassisori	0.09	8	0.93	$G = 0.25 + 0.36I$	225	9	<0.01	0.96
Harris and Paffenhöfer (1976)								
Pseudocalanus: NI to CI	9.65	1	0.06	$G = 0.72 + 0.09I$	4	2	0.19	0.65
CI to CIII	2.64	1	0.23	$G = -0.87 + 0.21I$	4	2	0.17	0.70
CIII to 50% adult	0.10	1	0.94	$G = -0.27 + 0.14I$	5	2	0.16	0.71
Temora: NI to CI	1.07	1	0.48	$G = 0.31 + 0.14I$	15	2	0.06	0.88
CI to CIII	1.93	1	0.30	$G = 1.00 + 0.21I$	19	2	0.05	0.90
CIII to 50% adult	−36.98	1	0.02	$G = 6.22 + 0.09I$	1	2	0.40	0.36
Conover and Lalli (1974)	−0.92	5	0.40	$G = 0.03 + 0.56I$	35	6	<0.01	0.85
Reeve (1963)								
5 d old	22.69	1	0.03	$G = 0.00 + 0.25I$	48	2	0.02	0.96
10 d old	−0.33	1	0.80	$G = -0.00 + 0.48I$	7	2	0.12	0.78
15 d old	−2.59	1	0.23	$G = 0.02 + 0.35I$	31	2	0.03	0.94
20 d old	−34.29	1	0.02	$G = 0.03 + 0.48I$	31	2	0.03	0.94
25 d old	−0.68	1	0.62	$G = 0.18 + 0.14I$	1	2	0.42	0.33
30 d old	6.39	1	0.10	$G = 0.08 + 0.30I$	26	2	0.04	0.93
35 d old	−0.31	1	0.81	$G = 0.08 + 0.18I$	16	2	0.06	0.89
40 d old	9.54	1	0.07	$G = 0.07 + 0.15I$	6	2	0.13	0.75
45 d old	0.84	1	0.55	$G = 0.06 + 0.10I$	8	2	0.11	0.79
50 d old	0.42	1	0.74	$G = -0.06 + 0.22I$	370	2	<0.01	0.99
55 d old	−1.48	1	0.38	$G = 0.08 + 0.14I$	1	2	0.39	0.37
Elliott (1975a, b)								
12-g group: 5.6°C	0.49	1	0.71	$G = -0.51 + 0.20I$	118	2	<0.01	0.98
9.5°C	−1.26	1	0.43	$G = -0.62 + 0.17I$	66	2	0.01	0.97
12.8°C	−2.80	2	0.11	$G = -0.83 + 0.14I$	39	3	<0.01	0.93
15.0°C	−3.34	2	0.08	$G = -1.13 + 0.12I$	46	3	<0.01	0.94
50-g group: 5.6°C	−21.20	1	0.03	$G = -0.32 + 0.19I$	2668	2	<0.01	>0.99
7.1°C	−0.99	1	0.50	$G = -0.39 + 0.18I$	255	2	<0.01	0.99
9.5°C	−1.98	1	0.30	$G = -0.38 + 0.14I$	139	2	<0.01	0.98
12.8°C	−2.11	2	0.17	$G = -0.44 + 0.12I$	78	3	<0.01	0.96
15.0°C	−9.86	2	0.01	$G = -0.55 + 0.09I$	31	3	0.01	0.91
17.8°C	−3.70	2	0.06	$G = -0.51 + 0.04I$	6	3	0.08	0.69
19.5°C	0.74	1	0.60	$G = -0.76 + 0.11I$	37	2	0.03	0.95
90-g group: 5.6°C	0.83	1	0.56	$G = -0.24 + 0.16I$	286	2	<0.01	0.99
9.5°C	−1.87	1	0.31	$G = -0.28 + 0.12I$	146	2	<0.01	0.99
12.8°C	−2.30	2	0.15	$G = 0.40 + 0.11I$	33	3	0.01	0.92
15.0°C	−3.55	2	0.07	$G = 0.44 + 0.08I$	28	3	0.01	0.91
250-g group: 5.6°C	−1.86	1	0.31	$G = -0.17 + 0.15I$	457	2	<0.01	>0.99
9.5°C	−1.22	1	0.44	$G = -0.20 + 0.12I$	61	2	0.02	0.97
12.8°C	−2.00	2	0.18	$G = -0.29 + 0.10I$	41	3	<0.01	0.93
15.0°C	−4.74	2	0.04	$G = -0.38 + 0.08I$	31	3	0.01	0.91
Corner et al. (1976)	−1.43	54	0.16	$G = -4.91 + 0.77I$	80	55	<0.01	0.59
Paulson (1980)								
Brook trout	−0.05	13	0.96	$E = 5.48 + 14.78I$	85	14	<0.01	0.86

TABLE 1. (*Continued*)

Author (case)	Test of quadratic term			Regression predicted[a]				
	t statistic	df MSE	*P*	Equation	*F* statistic	df MSE	*P*	r^2
Rainbow trout	−1.61	15	0.13	$E = 6.0 + 9.9I$	44	16	<0.01	0.73
Gerking (1955)								
Growth (nitrogen)	0.09	17	0.93	$G = -3.01 + 0.42I$	113	18	<0.01	0.86
Defecation (dry wt)	−0.43	17	0.68	$F = 0.04 + 0.05I$	101	18	<0.01	0.85
Kausch and Zimmerman (1976)								
Protein growth	0.01	15	0.99	$G = -0.99 + 0.36I$	203	16	<0.01	0.93
Caloric growth	−0.78	15	0.45	$G = -0.94 + 0.45I$	205	16	<0.01	0.93
Checkley (1980)	−4.96	41	<0.01	$G = 0.41I - 0.41X(I - 0.72)$; $X = 0$ for $I \le 0.72$, 1 for $I > 0.72$				
Lasker (1960, 1966)	1.24	29	0.22	$A_t = -7.93 + 0.40I$	144	30	<0.01	0.83
Sorokin and Panov (1965)								
Assimilation	−1.59	3	0.21	$A_s = 0.00 + 68I$	5197	4	<0.01	>0.99
Respiration of diet	−1.87	3	0.16	$R_f = 0.01 + 0.08I$	33	4	<0.01	0.89
Defecation	−0.37	3	0.72	$F = -0.01 + 0.24I$	761	4	<0.01	0.99
Sorokin (1966)	−4.58	3	0.02	$A_s = 0.43I - 0.43X(I - 58)$; $X = 0$ for $I \le 58$, 1 for $I > 58$				
Brett et al. (1969)								
5°C	−4.69	1	0.13	$G = -0.15 + 0.24I$	50	2	0.02	0.96
10°C	−10.83	2	<0.01	$G = -0.33 + 0.31I$	235	3	<0.01	0.99
15°C	−2.65	2	0.12	$G = -0.58 + 0.35I$	464	3	<0.01	0.99
20°C	3.09	2	0.09	$G = -0.54 + 0.24I$	289	3	<0.01	0.99
Kersting (1978)	−0.049	13	0.63	$G = -0.01 + 0.21I$	32	14	<0.01	0.70

[a] Fits of nonlinear regressions are compared in the text.

groups within a data set, I attributed this to experimental variation and fitted linear regressions to all the groups. If I fitted linear regressions to the data, I conducted an analysis of covariance (ANCOVA, Snedecor and Cochran 1967) to fit the regressions. Because the ANCOVA analyses are not central to the scope of the paper, the statistics are reported elsewhere (Condrey 1981), the exception being the reanalyses of Dawes' and of Elliott's data, which are reported here.

I report on all the published data I have found in which an author provides data on growth and ingestion at ingestion rates that are not limited to satiation or a single fixed percentage of the body weight, and where there are sufficient data to test for a quadratic term.

I use the term "absorption" to denote the transport of ingested material across the gut wall. Assimilation is used to denote the incorporation into tissue of absorbed material. Excretion denotes the release of absorbed or assimilated material from the body. Respiration is the release of excreted material as CO_2. Defecation denotes release from the anus of unabsorbed material.

Results

Accepted Models and Concepts Inconsistent with Blackman Kinetics

Paloheimo and Dickie — Paloheimo and Dickie (1966) said that "... published data on growth and feeding of fishes shows that when fish are fed on one type of food, the logarithm of the gross growth efficiency (log K) decreases with increase in ration ... (and that) ... for a number of species ... this relationship is adequately described by the linear equation

$$(2) \quad \log K = -a - bR"$$

where R is the ration intake (=ingestion, beginning at some ingestion rate greater than that supporting maintenance) and a and b are constants. Equation 2 predicts a curvilinear decline in the rate of increase in growth rate, as ingestion is increased from some level above maintenance to satiation. As such, it is at variance with Blackman kinetics. Only two of the data sets used by Paloheimo and Dickie in deriving Eq. 2 contained information on fish fed at ingestion rates other than satiation or a fixed percentage of their body weight: Dawes (1931a, b) and Gerking (1955).

In their analyses of Dawes' data on the plaice *Pleuronectes platessa*, Paloheimo and Dickie deleted those fish fed at "maintenance" levels and divided the rest of the data ("intermediate" and "maximum" levels) into two temperature series, ascending and descending. The correlation they noted between log K and ingestion was highly significant and negative for fish in the ascending temperature series but significant and positive with the descending group. Thus one group was consistent with their model, whereas the other was not.

I have examined all of Dawes' (1931a, b) data on plaice. The ANCOVA denoted a significant interaction of ingestion·sex·year·place ($P = 0.015$). The quadratic term was significant in three of the eight possible cases: twice positive and once negative (Table 1). For consistency, I fitted linear

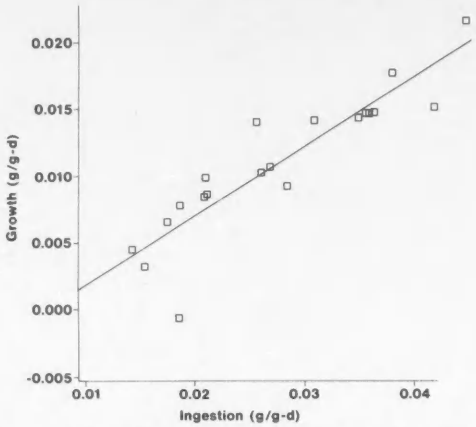

FIG. 1. Relationship between growth and ingestion for bluegill, *Lepomis macrochirus*, fed larvae of the mealworm *Tenebrio molita* (data from Gerking 1955): $G = -0.01 + 0.44 \cdot I$, ($P < 0.01$).

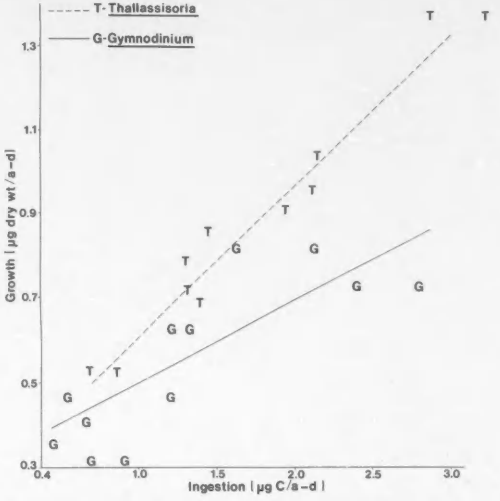

FIG. 2. Relationship between growth and ingestion in the copepod *Calanus helgolandicus* feeding on either the diatom *Thalassiosira fluviatilis* or the dinoflagellate *Gymnodinium splendens* (data from Mullin and Brooks 1970). Regression statistics are given in Table 1.

regressions to all the data. The fit of these regressions is consistent with Blackman kinetics and also with Dawes' (1931c) statistical analysis of his data.

Paloheimo and Dickie divided Gerking's dry weight data on the growth and ingestion of the bluegill *Lepomis macrochirus* into two groups (summer and fall) and deleted his data on fish fed at the lowest rate (1.16%/d). They found a significant relationship between log K and R for the summer group, but not for the fall group.

I have repeated their analyses. My results are numerically similar, but statistically dissimilar, to theirs for summer fish. I obtain a coefficient of correlation of -0.66 between log K and R. This is similar to the value reported in Paloheimo and Dickie, but dissimilar in that it is not significant at the 95% level ($P = 0.075$). Additionally I computed a coefficient of multiple correlation of 0.71 of log K on ration and weight, which is similar to that reported by Paloheimo and Dickie of 0.72. However, my coefficient of multiple correlation is not significant ($P = 0.17$). Rounding error accounts for the first discrepancy, because a coefficient of correlation $\geqq 0.666$ is significant with 7 df. (Snedecor and Cochran 1967, p. 557). The second discrepancy probably was caused by an unintentional error in consulting a statistical table, as a coefficient of multiple correlation of 0.72 is significant with 6, but not 5 df. I suggest that these errors led Paloheimo and Dickie to conclude erroneously that Gerking's data for summer fish were consistent with their model.

I plotted all of Gerking's dry weight data on growth and ingestion (starvation to satiation) in Fig. 1. A linear relationship is predicted. The quadratic term is not significant (Table 1). Gerking's dry weight data on sunfish appear to be consistent with Blackman kinetics.

Mullin and Brooks (1970) noted a negative correlation between growth efficiency and the total amount of food ingested by the copepod *Calanus helgolandicus*. This appears to be consistent with the Paloheimo and Dickie model and inconsistent with Blackman kinetics. However, as I show below, this is a result of the use of different units in measuring growth and ingestion.

I plotted estimates of growth and ingestion derived from Mullin and Brooks (1970) in Fig. 2. A linear regression is fitted to the data on each copepod, as there was no significant quadratic term (Table 1). Note that micrograms dry weight of animal is regressed against micrograms carbon ingested and that a positive "y" intercept is predicted. These positive intercepts are likely a result of a change in the ratio of carbon to total dry weight of the animals with ingestion rate (e.g. Kausch and Zimmerman 1976). Regardless, they are the reason the data appear to be consistent with the Paloheimo and Dickie model. The fit of the linear regressions suggests that the data are consistent with Blackman kinetics.

Harris and Paffenhöfer (1976) attempted to fit the Paloheimo and Dickie model to their data on the growth efficiency and ingestion rates of the copepods *Temora longicornis* and *Pseudocalanus elongatus*. They found significant correlations in only one of the six possible cases. They suggest that this lack of significant fit may be a function of the limited data, while Corket and McLaren (1978) suggest that it resulted "from unnecessary grouping into reduced sample size." I plotted Harris and Paffenhöfer's growth and ingestion measurements for the two copepods (Fig. 3). The quadratic term is significant only at the last development stage reported with *T. longicornis* (Table 1). For consistency, I fitted the data to linear regressions. The fit of the regressions is consistent with Blackman kinetics.

Conover and Lalli (1974) measured growth and ingestion in the swimming gastropod *Clione limacina*. They did not obtain a "significant slope whether log K (Eq. 2) was plotted against size of predator (*Clione*), amount of prey ingested (*Spiratella*), or mean size of prey." They suggest that "a negative

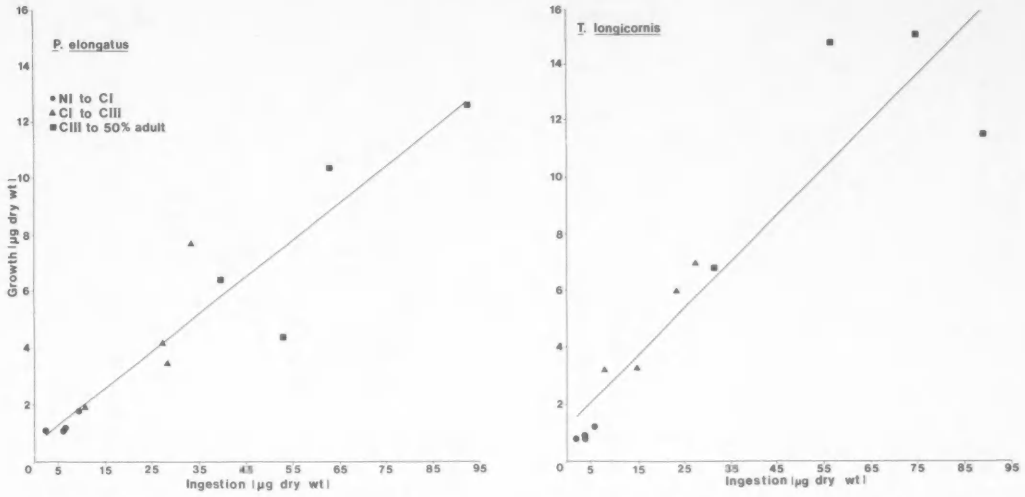

FIG. 3. Relationship between growth and ingestion in three stages of the copepods *Temora longicornis* and *Pseudocalanus elongatus* feeding on the diatom *Thalassiosira rotula* (data from Harris and Paffenhöfer 1976): $G = 1.20 + 0.17 \cdot I$, ($P < 0.01$); and $G = 0.60 + 0.13 \cdot I$, ($P < 0.01$).

slope for the *K*-line would result if the animals were grown with a constant size of food." I plotted the gastropod's rate of growth versus its rate of ingestion (Fig. 4). A linear regression is fitted to the data as the quadratic term is not significant (Table 1). The fit of the regression to the data is consistent with Blackman kinetics.

Reeve — Reeve (1963) examined the cumulative growth efficiency of the fairy shrimp *Artemia salina*. He noted "... a definite peak (cumulative growth) efficiency which occurs about the time of inflection in the growth (in length) rate curve ... (and) ... that the peak is highest in the lowest food levels, although the rate of growth is slower, the onset of sexual maturity is later, and the brood and the ultimate size of the animals are smaller." His interpretation is at variance with Blackman kinetics.

When I plotted Reeve's estimates of incremental growth and ingestion versus age, it appeared to me that experimental variation may have caused the apparent peaks in cumulative efficiency. For example, note the abrupt peak in growth rate at age 25 d of animals held at the second lowest algal concentration (Fig. 5).

I examined individual regressions and plots of Reeve's data by age. In only two of the 11 cases was the quadratic term significant: once positive, once negative (Table 1). I attributed this behavior to experimental variation and fitted the data to linear regressions (Fig. 6). Parallel regressions are used, because the ANCOVA analyses suggested that the significant effect of age on a total line was caused by a difference between animals that were 20–30 d old and those of the other ages. I suggest that the fit of the parallel regressions to the data is consistent with Blackman kinetics.

Elliott — Elliot (1975a, b) measured the growth of brown trout, *Salmo trutta*, as a function of weight, temperature, and ration (starvation to satiation). He examined his data at the various weight and temperature levels in plots of growth (g wet wt/g wet wt-d) versus ingestion rate (mg dry wt/d). "As a generally suitable linear transformation could not be found, smooth curves were fitted by eye" (Elliott 1975b).

Elliott's interpretation is not necessarily at variance with Blackman kinetics. If there is a linear relationship between growth rate and ingestion rate, it may appear curvilinear when only one rate is expressed in terms of body weight and the experiment has resulted in substantially different body weights for animals fed different rations.

I examined Elliott's published data in plots of growth and ingestion rates where both were expressed as ratios of the average body weight. The quadratic term was significant and negative in only 3 of the 18 cases (Table 1). I attribute this behavior to experimental variation. I fitted linear regressions to each temperature–initial body weight group because there was significant interaction of temperature·ingestion ($P < 0.01$) and initial weight·ingestion ($P < 0.01$). The fit of these linear regressions is consistent with Blackman kinetics (Fig. 7).

Corner et al. — Corner et al. (1976) fitted a curve of the form

$$(3) \quad G = [a \cdot (I - I_m)]/[b + (I - I_m)]$$

to his growth and ingestion data on the copepod *C. helgolandicus*. In Eq. 3, I_m is the maintenance ingestion rate and a and b are constants. Their model is at variance with Blackman kinetics as it predicts a curvilinear decline in the rate of increase in growth rate with increasing ingestion rate.

I tested the fit of Blackman kinetics to their data. The quadratic term was not significant (Table 1). I also compared

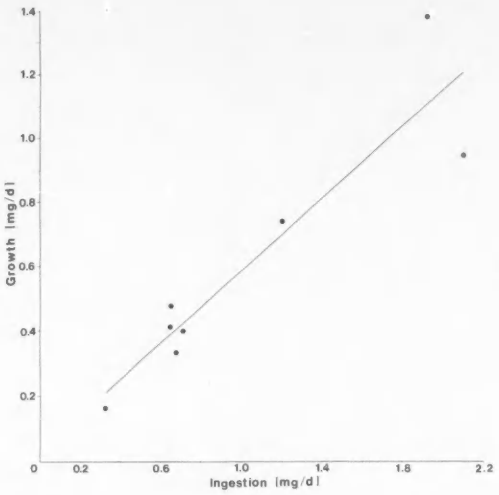

FIG. 4. Relationship between growth and ingestion for the naked pteropod *Clione limacina* feeding on the sea butterflies (shelled pteropods) *Spiratella retroversa* and *S. helicina* (data from Conover and Lalli 1974). Final weights of the animals were estimated by averaging the reported measured and calculated final weights. Regression statistics are given in Table 1.

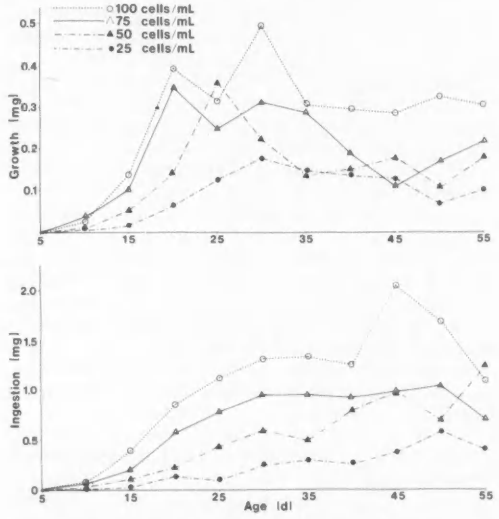

FIG. 5. Incremental (5 d) rates of growth and ingestion for the fairy shrimp *Artemia salina* feeding at four concentrations of the diatom *Phaeodactylum tricornutum* (data from Reeve 1963).

my linear fit with that of their equation. Though Eq. 3 did provide a lower sum of squares than the linear regression, 1158 compared with 1195, this was still higher than that obtained with the quadratic equation 1151. Within the statistical guidelines used in this paper, I conclude that Eq. 3 does not provide a statistically better fit than does a simple linear regression consistent with Blackman kinetics. Equations are compared in Fig. 8.

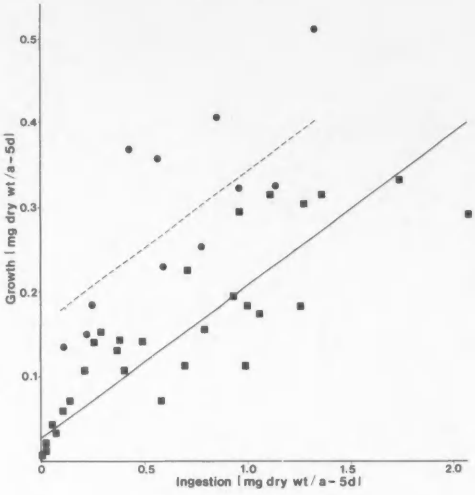

FIG. 6. Relationship between growth and ingestion for the fairy shrimp *Artemia salina* feeding at four concentrations of the diatom *Phaeodactylum tricornutum* (data from Reeve 1963). Parallel regressions are those predicted for animals (- - -) of ages 20–30 d and for animals (—) of ages 5–15 and 35–55 d: $G = 0.16 + 0.18 \cdot I$ and $G = 0.03 + 0.18 \cdot I$, ($P < 0.01$).

(v) *Paulson* — Paulson (1980) related excretion to ingestion for rainbow trout (*Salmo gairdneri*) and brook trout (*Salvelinus fontinalis*) with an exponential relationship

$$(4) \quad E = a \cdot 10^{(Rb_1 + Tb_2)} \cdot W^{b_3}.$$

He used a log transformation and generated multiple-regression equations relating ammonia excretion (E) to ration = ingestion (R), temperature (T), and body weight (W) separately for the two fish. The published portion of his data suggests that a linear model would also provide a good fit.

I tested the fit of Paulson's model against a linear interpretation of his published data. With Paulson's model I obtained multiple coefficients of determination of 0.80 for brook trout and 0.58 for rainbow trout. The linear regressions of specific excretion rate against specific ingestion rate provided a better fit, with coefficients of determination of 0.86 and 0.73 for brook and rainbow trout. The quadratic term was not significant with either fish (Table 1). As my ANCOVA analysis showed no effect of species on a total line, a single regression is plotted in Fig. 9. The data are consistent with the simplest interpretation of Blackman kinetics — that of a linear relationship between excretion and ingestion as long as growth rate is limited by ingestion rate.

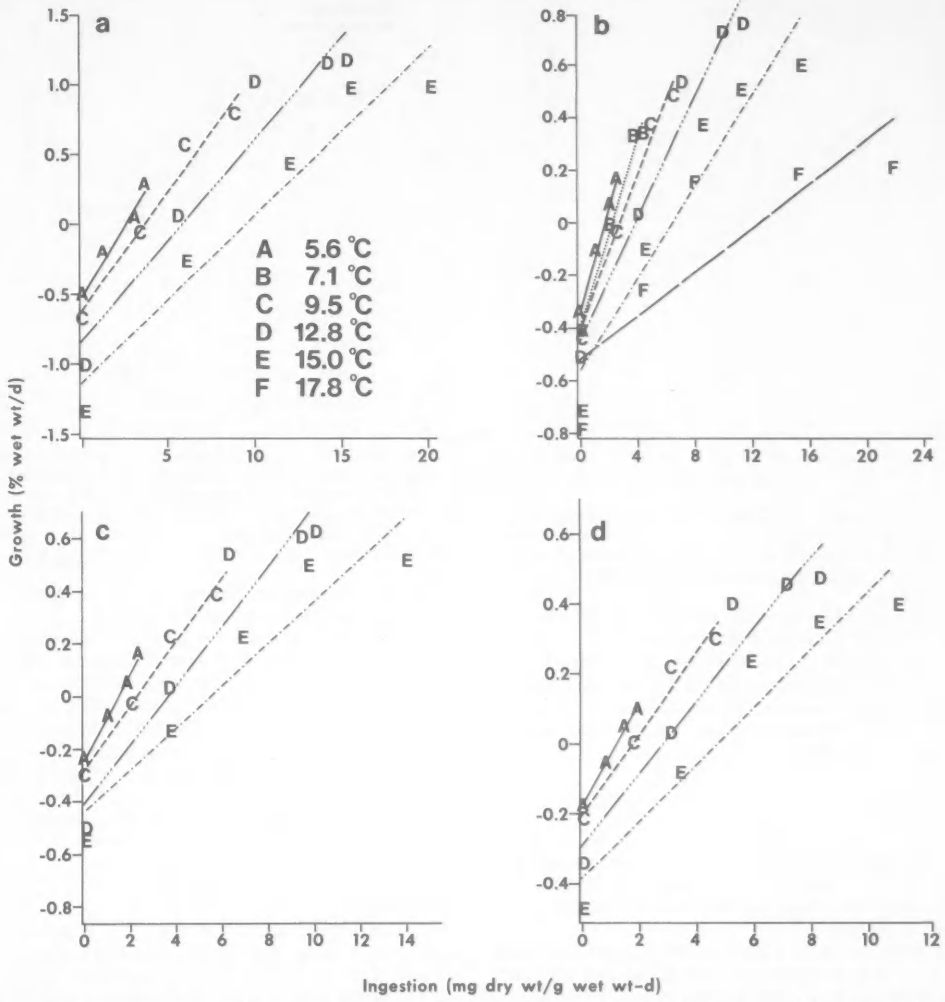

FIG. 7. Relationship between growth and ingestion in brown trout, *Salmo trutta*, as a function of temperature and initial body weight: (a) ~12 g wet wt, (b) ~50 g wet wt, (c) ~90 g wet wt, and (d) ~250 g wet wt (data from Elliott 1975a, b). Regression statistics are given in Table 1.

AUTHORS WHO HAVE INDEPENDENTLY APPLIED BLACKMAN KINETICS

I have found three instances in which authors report a linear dependence of growth rate on ingestion rate for an aquatic animal fed at levels ranging from starvation to satiation: Dawes (1931c) with the data on plaice he reports in Dawes (1931a, b), Gerking (1955) with his nitrogen data for bluegill sunfish, and Kausch and Zimmerman (1976) with whitefish, *Coregonus fera* (starvation to satiation). I have found one instance where an author applied a rectilinear relationship to his growth rate data: Checkly (1980) with egg production of the copepod *Paracalanus parvus*.

I reported on Dawes' data earlier in this paper. I have reexamined Gerking and Kausch-Zimmerman. In both cases the quadratic term is not significant and the fit of the data about the regressions is consistent with Blackman kinetics (Table 1).

I have reexamined Checkly's data. The quadratic term is highly significant (Table 1). The residual sum of squares was lower when the data were fit to Blackman, than to Monod, kinetics: 0.131 as compared with 0.149 (corrected total sum

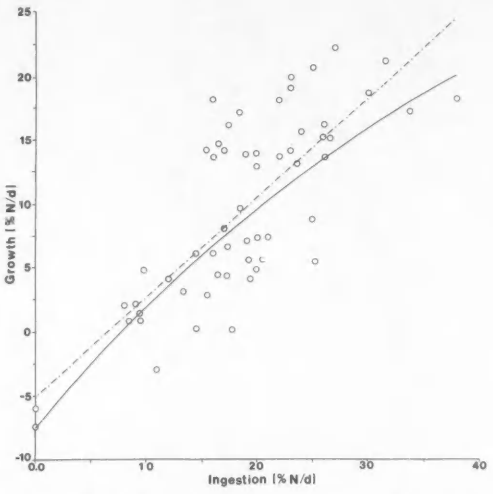

FIG. 8. Relationship between growth and ingestion for the copepod *Calanus helgolandicus* feeding on Stage II nauplii of the barnacle *Elminius modestus* (data from Corner et al. 1976). The curve drawn is from Corner et al. Linear regression statistics are given in Table 1.

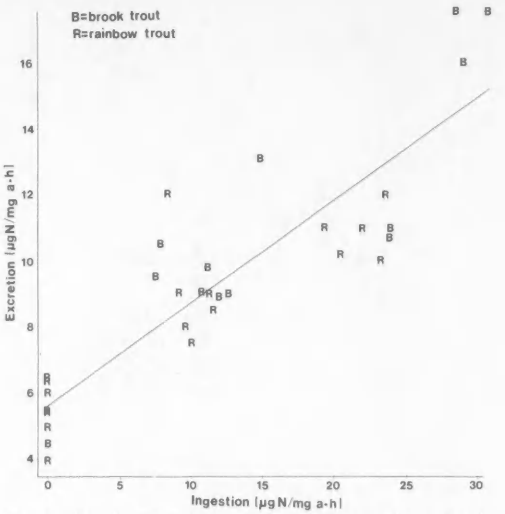

FIG. 9. Relationship between nitrogen excretion and ingestion for brook trout, *Salvelinus fontinalis*, and rainbow trout, *Salmo gairdneri* (data from Paulson 1980): $E = 5.62 + 13.06 \cdot I$, ($P = 0.01$).

of squares = 0.482 with 43 df). The data remained consistent with Blackman kinetics in the single- and double-reciprocal plots. For example, the double-reciprocal plot, Fig. 10, suggests that egg production was saturated at the higher ingestion rates. The sharp inflection at I_x characteristic of Blackman kinetics allows the predicted equation to follow the data through the transformation.

OTHER DATA SETS

I plotted Lasker's (1960, 1966) data for the euphausiid shrimp *Euphausia pacifica*, in Fig. 11. A linear relationship of assimilation to ingestion is suggested. The quadratic term is not significant (Table 1). The data are consistent with Blackman kinetics.

I plotted Sorokin and Panov's (1965) data on bream larvae in Fig. 12. The relationship between assimilation and ingestion, as well as those between losses of the diet (as CO_2 and feces) and ingestion, are markedly linear. Linear regressions were fitted to the data. In all cases, the quadratic term was not significant (Table 1). The data are consistent with the simplest interpretation of Blackman kinetics — that growth is a linear function of ingestion rate at $I \leqq I_x$ because the absorption and assimilation efficiencies are constants as long as growth is limited by ingestion rate.

I plotted Sorokin's (1966) data on assimilation and ingestion for the water flea *Daphnia pluex* in Fig. 13. The quadratic terms is highly significant (Table 1). I fitted Blackman and Monod kinetics to his data and examined the fit through the single- and double-reciprocal plots. The residual sum of squares was lower when the data were fit to Blackman, than to Monod, kinetics; 21.27 as compared to 76.07 (corrected total sum of squares = 194 with 5 df). The data remained consistent with Blackman kinetics through the double- and single-reciprocal plots, e.g. Fig. 13.

I plotted the data of Brett et al. (1969) on growth and ingestion for fingerling sockeye salmon, *Oncorhynchus nerka* (Group "B" fish), in Fig. 14. The quadratic term was significant only for fish at 10°C (Table 1). For consistency I fitted linear regressions to the data. The fits of the data to the regressions are consistent with Blackman kinetics.

I plotted Kersting's (1978) data on the growth and ingestion of the water flea *Daphnia magna* in Fig. 15. The quadratic term was not significant (Table 1). The fit of the data to the regression is consistent with Blackman kinetics.

Discussion

All of the data examined are consistent with Blackman kinetics. In 13 of the 15 papers where growth or assimilation rate was measured, the data were consistent with the hypothesis of a simple linear dependence of growth (or assimilation) rate on ingestion rate. In the other two cases, the data were consistent with the hypothesis of a rectilinear relationship in which growth (or assimilation) was linearly dependent on ingestion at ingestion rates less than a given value. Above this level, growth was independent of ingestion.

In three papers, data were provided on defecation, excretion, and/or respiration of absorbed material that had not been assimilated. In all these cases, the data were consistent with the hypothesis of a simple linear dependence of the measured parameter on ingestion.

The analyses suggest, then, that maximum growth and maximum growth efficiency will be attained at maximum ingestion rate (satiation) unless some other factor affecting growth is suboptimal.

It is possible that an inflection at I_x in growth's response to

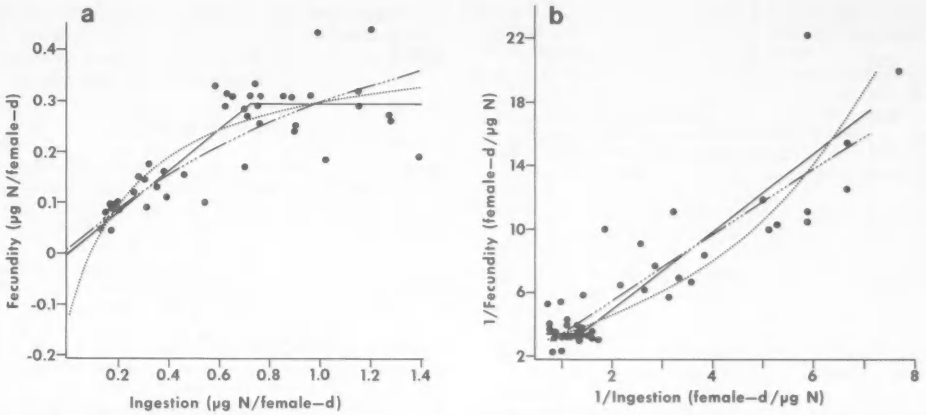

FIG. 10. Comparing the fit of Blackman and Monod kinetics with data on fecundity and ingestion of the copepod *Paracalanus parvus*: (a) untransformed data and (b) the double-reciprocal transformation (data from Checkley 1980). Lines drawn are the solution to the Blackman (—) and Monod (- - -) equations fit to the untransformed data and Monod kinetics fit the double-reciprocal transformed data (— - - -). Regression statistics for Blackman kinetics are given in Table 1.

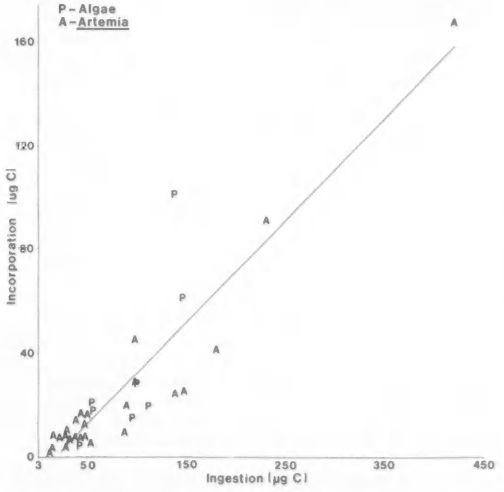

FIG. 11. Relationship between assimilation = incorporation and ingestion for the euphausiid shrimp *Euphausia pacifica* feeding on either the green alga *Dunaliella primolecta* or nauplii of the fairy shrimp *Artemia salina* (data from Lasker 1960, 1966). Regression statistics are given in Table 1.

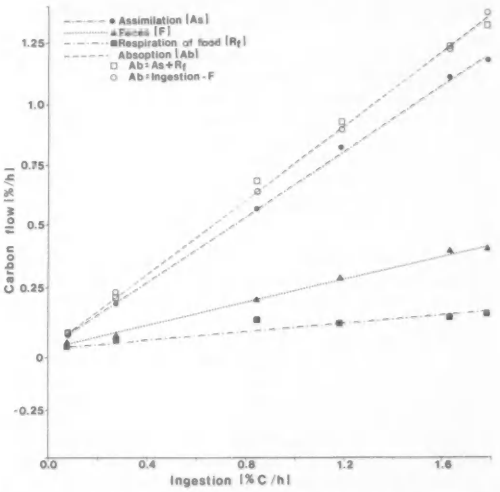

FIG. 12. Carbon flow through bream larvae feeding on the water flea, *Bosmina longirostris* (data from Sorokin and Panov 1965). Regression statistics are given in Table 1.

ingestion will be due to a saturation of the animal's inherent (genetic) ability to incorporate ingested diet at $I > I_s$. However, this cannot be assumed. I am not aware of any data which would support this hypothesis. Recall that all of the data examined here which had been used to suggest such a pattern have been shown to be consistent with a simple linear dependence of growth rate on ingestion rate.

The sharp inflection point predicted at I_s is not consistent with our ability to estimate population responses. Curnow (1973) has shown that if the individuals within a population exhibit a rectilinear response as discussed by Blackman, and the response is measured on the population, then the individual differences in where the inflection points occur (e.g. I_s) will result in a curvilinear transition as is suggested by the data in Fig. 13a. I have not used his treatment in this paper for two reasons. First, it requires a constant assimilation to ingestion ratio among the individuals within the population. I do not feel that that is a valid assumption for the conditions examined

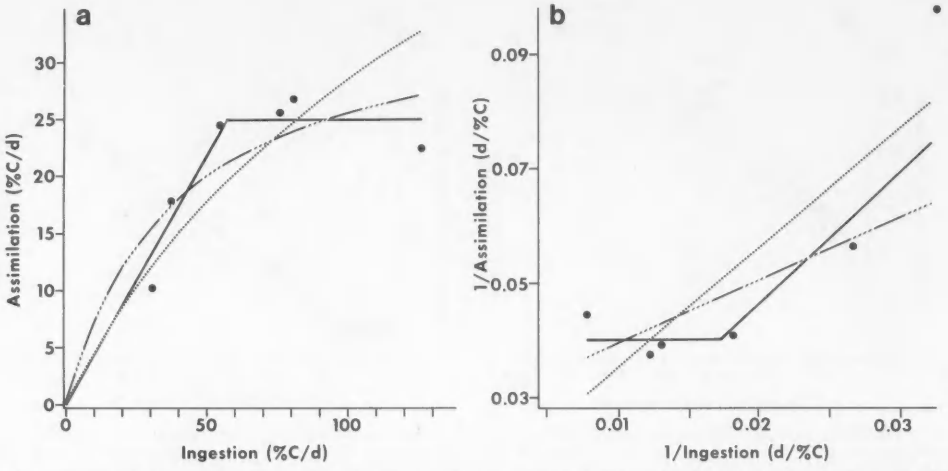

FIG. 13. Comparing the fit of Blackman and Monod kinetics to data on assimilation and ingestion for the water flea *Daphnia pulex* feeding on the green alga *Chlorococcum*: (a) untransformed data and (b) the double-reciprocal transformation (data from Sorokin 1966). Lines drawn are the solutions to the Blackman (—) and Monod (— - - -) equations fit to the untransformed data and to the Monod equation fit to the double-reciprocal transformed data (- - -). Regression statistics for Blackman kinetics are given in Table 1.

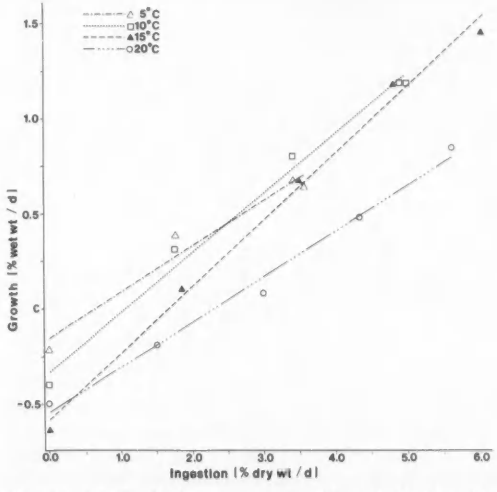

FIG. 14. Relationship between growth and ingestion for fingerling sockeye salmon, *Oncorhynchus nerka*, raised at four temperatures (data from Brett et al. 1969). Regression statistics are given in Table 1.

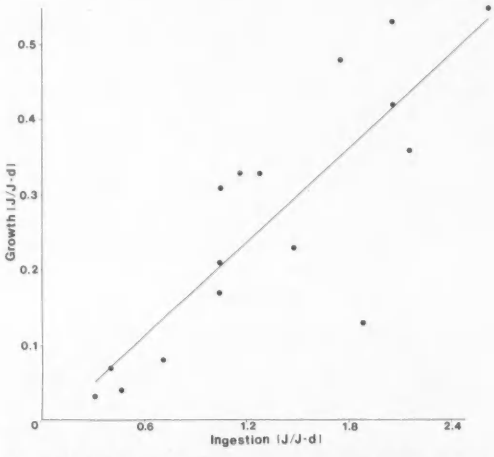

FIG. 15. Relationship between growth and ingestion of the water flea *Daphnia magna* feeding on the green alga *Chlorella vulgaris* (data from Kersting 1978). Regression statistics are given in Table 1.

here and did not see the utility of applying a rather complex, and still inadequate, model. Second, we may find that I_x is not attained because of an inherent (genetic) inability of the animal to assimilate diet as efficiently at high ingestion rates as it does at low rates. If this is true, then I_x is a transient parameter dependent upon the suboptimal concentration of another (and external) factor affecting growth.

If these results reflect reality, then there is the possibility of a commonality in the processes governing growth in all living organisms. I have shown that Monod's (1942) data on substrate-limited growth of bacteria is consistent with Blackman kinetics. I show here that all the data I have found on ingestion-limited growth of aquatic animals is consistent with Blackman kinetics. Fisher (1980) has shown a rectilinear relationship between fecundity of chickens and concentration of limiting amino acid in the diet which is consistent with Black-

man kinetics. And Morgan (1981) has demonstrated a linear dependence of growth on ingestion for rats which is consistent with Blackman kinetics. I suggest that the possibility of such a commonality deserves our careful study.

Acknowledgments

I am indebted to Dr Michael Landry for the initial insight he provided in pointing out that the data of Brett et al. on "Group B" fish were consistent with a straight line. I am also indebted to Drs Frieda Taub, Doug Chapman, Alan Seymor, Michael Landry, and Richard Strathman for their helpful discussions during the first phases of the work and to Dr James Geaghan for his statistical advice. Drs James Gosselink, James Kitchell, Daphne Fairbairn, Mr W. Barnes Johnson, and an anonymous referee made valuable suggestions on the manuscript.

This is report No. LSU-CEL-81-03 of the Coastal Ecology Laboratory, Center for Wetland Resources, Louisiana State University, Baton Rouge, LA. It results from research supported by the LSU and UW Sea Grant College Programs, sponsored by the National Oceanic and Atmospheric Administration, Department of Commerce.

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Hepatic Metallothionein in Rainbow Trout (*Salmo gairdneri*) as an Indicator of Metal Pollution in the Campbell River System

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ROCH, M., J. A. MCCARTER, A. T. MATHESON, M. J. R. CLARK, AND R. W. OLAFSON. 1982. Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River system. Can. J. Fish. Aquat. Sci. 39: 1596 – 1601.

Hepatic metallothionein was measured in livers of freshly killed rainbow trout (*Salmo gairdneri*) using differential pulse polarography. The fish were caught in metal-contaminated lakes of the Campbell River watershed and in a nearby control lake. The livers were analyzed for zinc, copper, and cadmium using atomic absorption spectrophotometry. High and low molecular weight protein fractions were separated by gel chromatography from liver cytosols and analyzed for metals. A downward trend from the most contaminated lake to the least was found in levels of zinc in the water, of cadmium and copper in high molecular weight fractions, and of copper in low molecular weight fractions and metallothionein. The concentration of metallothionein is a useful quantitative measure of the degree of exposure of fish to heavy metals.

Key words: metallothionein, rainbow trout, *Salmo gairdneri*; heavy metal pollution, sublethal exposures, mine wastes

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La métallothionéine hépatique a été mesurée par polarographie à impulsions différentielles dans des foies de truites arc-en-ciel (*Salmo gairdneri*) fraîchement tuées. Ces poissons provenaient de lacs pollués par métaux du bassin hydrographique de la rivière Campbell et d'un lac témoin avoisinant. Nous avons dosé le zinc, le cuivre et le cadmium dans ces foies par spectrophotométrie à absorption atomique. Les fractions de protéines de poids moléculaire élevé et faible ont été séparées des cytosols hépatiques par chromatographie sur gel, et la teneur en métaux a été déterminée. On observe une gradation descendante, depuis le lac le plus contaminé au lac le moins contaminé, des niveaux de zinc de l'eau, de cadmium et de cuivre dans les fractions à poid moléculaire élevé et de cuivre dans les fractions à faible poids moléculaire, et enfin de la métallothionéine. La teneur de cette dernière est une mesure quantitative utile du degré d'exposition des poissons aux métaux lourds.

Received February 17, 1982
Accepted July 30, 1982

Reçu le 17 février 1982
Accepté le 30 juillet 1982

VARIOUS approaches have been used to predict safe levels of heavy metals in water for fish. Some have involved the determination of growth and survival during long-term exposure (McKim and Benoit 1971; Benoit et al. 1976) and measurement of physiological and biochemical disturbances at sublethal levels of metals (Christensen 1975; Larsson et al. 1976).

Ideally, measurements of biological response should be based on an understanding of the intracellular mechanisms by which a fish responds to exposure to heavy metals. It would be especially useful if it could be shown that toxic effects of a metal are related to the perturbation of a particular key biochemical process. Measurement of the extent to which such an alteration had occurred in a given situation would provide a good indication of the significance of the toxic effect.

Although the key biochemical processes associated with heavy metal poisoning have yet to be identified, specific molecular effects have been noted following metal exosure. The low molecular weight protein metallothionein is synthesized in the livers of mammals on exposure to cadmium, copper, zinc, and mercury (Kägi and Nordberg 1979). Metallothionein-like proteins have also been shown to exist in

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Printed in Canada (J6821)
Imprimé au Canada (J6821)

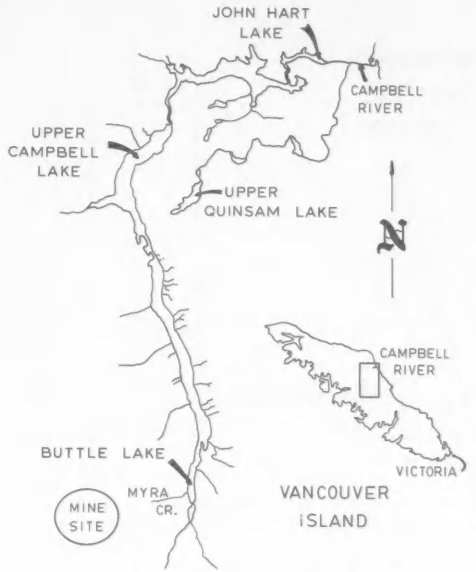


FIG. 1. Map of Vancouver Island and of the Campbell River drainage area showing the site of the mine and locations where samples of water and fish were collected. The mine is located at Myra Creek, South Buttle Lake.

teleosts, and the quantity of this protein increases following administration of cadmium (Olafson and Thompson 1974; Noël-Lambot et al. 1978; Overnell and Coombs 1979), mercury (Bouquegneau et al. 1975), zinc (Marafante 1976), and copper (McCarter et al. 1982). Purification and amino acid analysis of teleost cadmium metallothionein (Noël-Lambot 1978; Overnell and Coombs 1979) and copper metallothionein in coho salmon (*Oncorhynchus kisutch*) (McCarter et al. 1982) have proved the identities of these proteins.

Recently, we have shown (McCarter and Roch 1982) that coho salmon continuously exposed to sublethal levels of dissolved copper developed metallothionein concentrations in proportion to the logarithm of the concentration of copper to which the fish were exposed. These levels reached a plateau after 4 wk of exposure and provided a measure of sublethal response that could be related to the LC50.

Recent development and application of differential pulse polarography to the measurements of sulfhydryl activity of heat-denatured extracts of tissues (Olafson and Sim 1979) have allowed rapid and sensitive determination of metallothioneins in tissues. These authors have demonstrated the specificity of the technique for metallothionein and the absence of response to other sulfur-containing contaminants.

We have used the polarographic technique to determine whether rainbow trout (*Salmo gairdneri*) in the Campbell River drainage synthesized metallothionein in proportion to the extent of pollution of the water, and whether this parameter might have advantages over others, for example, levels of metal in tissues, as indicators of the extent of exposure of fish to metals.

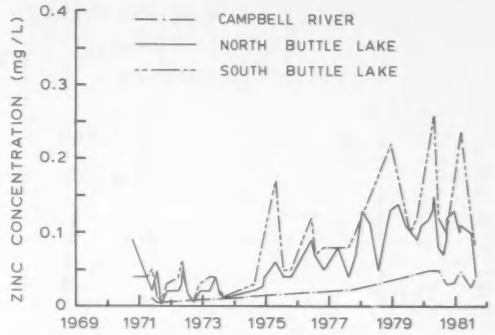


FIG. 2. Concentrations of Zn at 0–5 m in depth in waters of the Campbell River drainage area from January 1965 to December 1981.

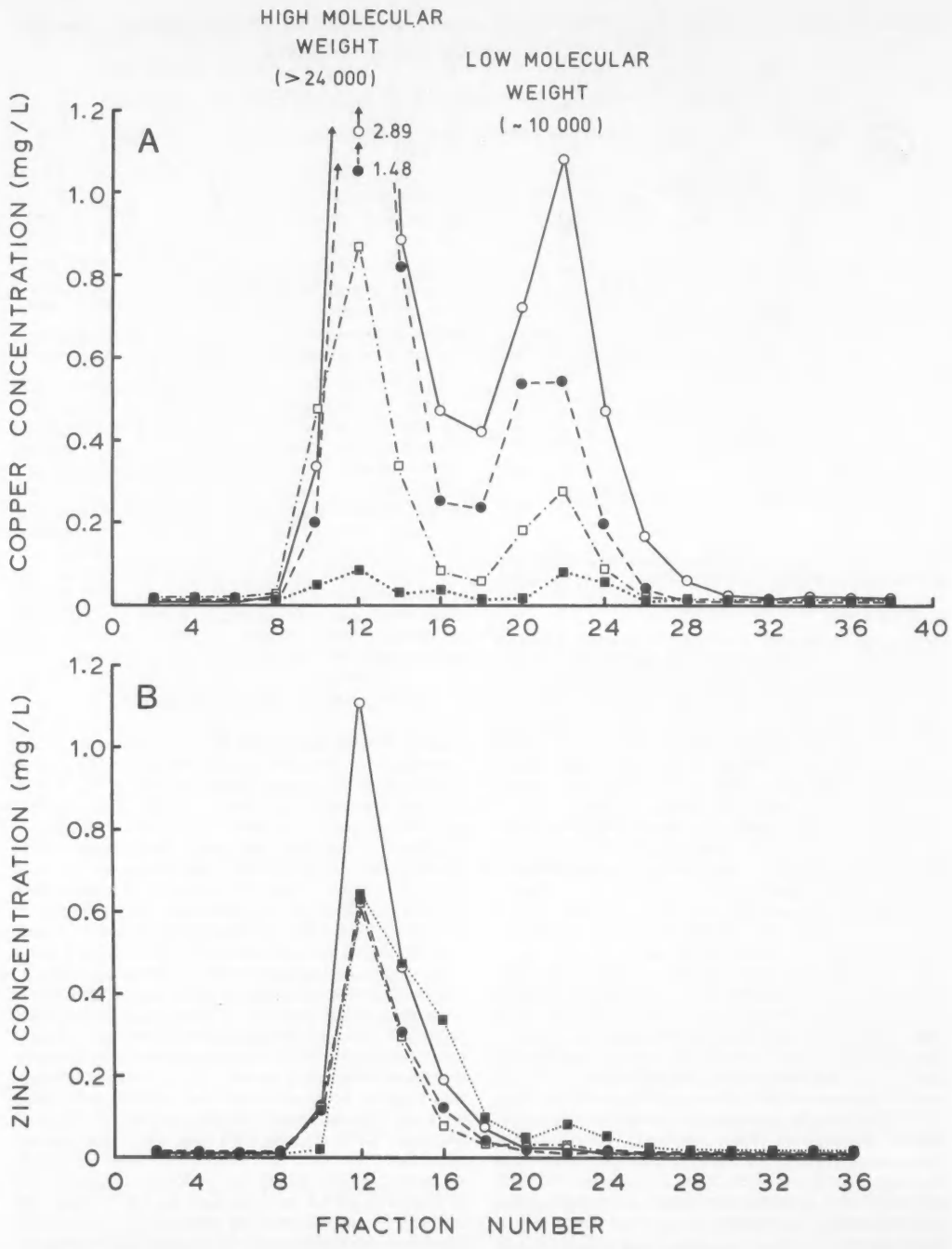
The Campbell River system (Fig. 1) is ideal for a field study of this sort because it provides a series of lakes containing dissolved metals. Metal contamination of surface and groundwater near a mine at the south end of Buttle Lake, and direct discharge of tailings to the lake have contributed dissolved metals, particularly zinc, to Buttle Lake and downstream locations. There has been a substantial and statistically significant ($P < 0.05$) increase in zinc levels over the period of the last 10 yr (Fig. 2) (Clark and Morrison 1982).

With this knowledge in hand, it should be possible to relate metallothionein levels to lethal toxicity of a combination of heavy metals as was done with coho salmon exposed to copper and thus, given a sample of wild trout, to obtain quantitative assessment of the toxicity of waters for trout.

Materials and Methods

Fish were collected in South Buttle, Upper Campbell, and Upper Quinsam lakes on August 25, 26, and 27, 1981, and at John Hart Lake (Fig. 1) on September 8, 1981, using gill nets. The fish were weighed, measured, and identified as to species. The fish were killed with a blow to the head and dissected to obtain the livers which were frozen immediately in dry ice. From each of the four sample locations, five trout ranging in size from 50 to 170 g were used. The correlations between metallothionein and fish weight, and copper in liver and fish weight are not significant ($P < 0.05$). The samples were kept frozen and transported to the University of Victoria in dry ice and subsequently stored in a freezer at −20°C.

Each liver was homogenized in 3 volumes of ice-cold 0.9% NaCl and 10 mmol Tris buffer, pH 8.6, with eight passes of a stainless steel/Teflon, Potter–Elvehjem homogenizer. Two aliquots of 0.2 mL were set aside; one for metallothionein determination and the other for heavy metal analysis. To obtain the cytosol fraction the remaining homogenate was centrifuged in a Sorvall RC5B centrifuge for 10 min at 5°C and 15 000 rpm in the SS34 rotor. The supernatant obtained from three fish at each location was pooled and applied to a Sephadex G-75 column (2.7 × 85 cm) and eluted with 10 mmol Tris, pH 8.6, in 15-mL fractions. The fractions were analyzed for copper, zinc, and cadmium by flame atomic absorption spectrophotometry (Varian AA/475). Elution of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LOW MOLECULAR WEIGHT (~10 000)
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B
ZINC CONCENTRATION (mg/L)
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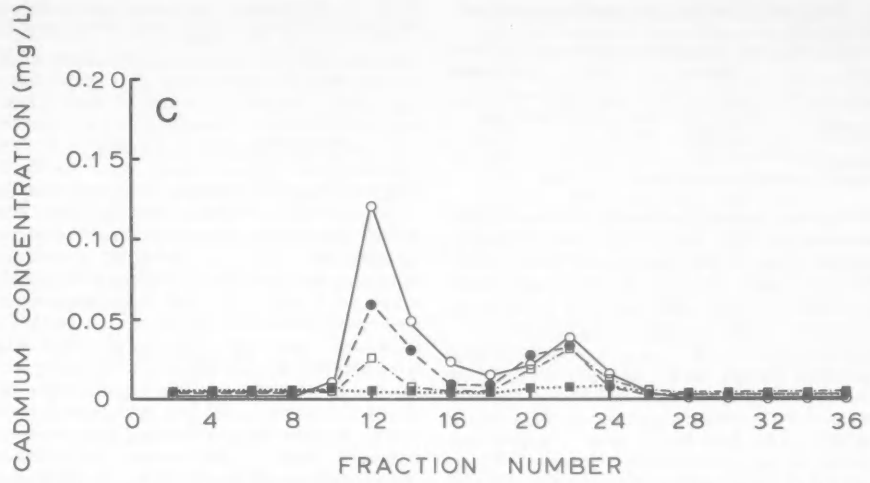

FIG. 3. Metals in fractions of liver cytosol from rainbow trout caught where indicated. Proteins were fractionated using Sephadex G-75. (—), South Buttle Lake; (---), Upper Campbell Lake; (·-·), John Hart Lake; and (···) Upper Quinsam Lake. A = copper; B = zinc; C = cadmium.

TABLE 1. Fiftieth percentile heavy metal concentrations (μg/L) during 1981. Tenth and 90th percentiles are given in parentheses.

Location		Copper	Zinc	Cadmium
South Buttle	(n = 36)	9 (3, 23)	170 (100, 250)	0.7 (0.5, 2.1)
Upper Campbell	(n = 32)	2 (1, 4)	60 (35, 80)	<0.5 (0.5, 0.8)
John Hart	(n = 30)	1 (1, 11)	50 (9, 60)	<0.5 (0.5, 1.2)
Upper Quinsam	(n = 29)	1 (1, 2)	<5 (<5, <5)	<0.5 (0.5, 1.5)

the column with 0.1% Na_2 EDTA following application of four samples indicated that no appreciable quantities of metal had been retained on the column.

Aliquots of the original homogenate (0.2 mL) intended for metallothionein measurement were made up to 5 mL in 0.9% NaCl in 10 mmol Tris, pH 8.6, and heat denatured in a water bath at 80°C for 5 min. Denatured homogenates were kept in ice and filtered through a 0.45-μm filter.

Metallothionein in the filtrate was determined by polarography using a modification of the Brdička (1933) procedure described by Olafson and Sim (1979), standardized by using metallothionein isolated from the hepatopancreas of the crab *Scylla serrata* (Olafson et al. 1979; Lerch et al. 1981).

The other 0.2-mL aliquot of homogenate was digested in 1.0 mL of hot nitric acid and 0.15 mL of 50% H_2O_2, made up to 5 mL with distilled water and analyzed for copper, zinc, and cadmium by atomic absorption spectrophotometry. Analysis of National Bureau of Standards standard bovine liver gave 99.6 ± 5.4% recovery of copper (n = 15) and 100 ± 16.5% of zinc. Cadmium, added to the standard bovine liver was recovered with 99 ± 3% efficiency.

Water samples were collected at depths of 1−50 m using Van Dorn bottles, at sites close to those where fish were collected. The samples were passed through a membrane filter (Millipore, 0.45 μm), acidified and assayed for zinc by atomic absorption (flame), and for copper and cadmium by graphite furnace.

Results

METAL CONTENTS OF WATER AND LIVERS OF FISH

The concentrations of metals in the waters of the polluted system (Table 1) were in the order Zn > Cu > Cd and, at least for Zn, decreased with increasing distance downstream from the mine. In the nonpolluted lake, Upper Quinsam, the 50th percentile concentrations of metals were at the lower limits of measurement (Cd 0.5, Cu 1, and Zn 5 μg/L).

Despite the differences in concentrations of Zn between sites, no significant differences existed between the total Zn concentrations (Table 2) in the livers of fish caught at the south end of Buttle Lake and those caught in Upper Quinsam ($P < 0.05$). The levels of Cu and Cd were greater in livers of fish caught at South Buttle Lake than in those at Upper Quinsam ($P < 0.05$).

METAL CONTENTS OF SEPHADEX G-75 FILTERED LIVER CYTOSOL

As shown in Table 2, copper was the principal metal

TABLE 2. Heavy metal in rainbow trout liver homogenate (μg/g, $n = 5$, mean ± SD).

Location	Copper	Zinc	Cadmium
South Buttle Lake	539±124	168±50	19.4±6.1
Upper Campbell	496±238	161±20	15.9±7.0
John Hart	196±39	123±33	8.6±2.9
Upper Quinsam	35±14	123±21	4.0±0.6

present in the liver homogenates. It was also the predominant metal associated with liver proteins. Copper in both low and high molecular weight protein-containing fractions separated by gel filtration on Sephadex G-75 (Fig. 3A) showed a definite trend toward decreasing concentrations in proportion to distance downstream from the source of contamination.

Concentrations of zinc (Fig. 3B) in both low and high molecular weight proteins did not appear to differ among sample locations except for fish from South Buttle Lake: these had almost twice the zinc in the high molecular weight region than was evident in fish from other locations. Very little zinc was present in the low molecular weight proteins.

Concentrations of cadmium (Fig. 3C) in the high molecular weight proteins showed a decreasing trend with distance downstream from the mine site, but those in the low molecular weight proteins showed no evident trend.

METALLOTHIONEIN

The low molecular weight metal-containing peak had a high sulfhydryl content and molecular weight of 10 000. Metallothionein, isolated from this peak, accounts for all of the sulfhydryl content as determined by polarography (R. W. Olafson unpublished data).

Differential pulse polarographic determinations of heat-stable cysteine-rich proteins (metallothioneins) from liver homogenates showed a definite increase in concentration proportional to the levels of zinc in water (Table 3). The correlation coefficient was 0.967. Correlation coefficients between metallothionein and copper in high and low molecular weight fractions were 0.994 and 0.995, respectively ($P < 0.01$).

TABLE 3. Metallothionein in rainbow trout liver (nmol/g, $n = 5$).

Location	$\bar{x}$ ± SD
South Buttle Lake	269±23
Upper Campbell	164±37
John Hart	94±18
Upper Quinsam	58±14

Discussion

Methods presently used to assess hazard are based primarily on laboratory data evaluating lethal response and application of this information to a specific problem. For example, Chapman (1978a) determined 96-h LC50 concentrations for juvenile steelhead trout (*Salmo gairdneri*) to be 93, 17, and 1.3 μg metal/L for zinc, copper, and cadmium, respectively, in water of identical hardness with that of Buttle Lake (25 mg/L as $CaCO_3$). Based on the additive toxic effect of zinc and copper on rainbow trout (Lloyd 1961), one would predict that metal levels in South Buttle Lake are in excess of lethal levels. Nevertheless, rainbow trout, cutthroat trout (*Salmo clarki*), and Dolly Varden (*Salvelinus malma*) can be found in these waters.

Recent evidence (Chapman 1978b; Dixon and Sprague 1981; Buckley et al. 1982) shows that salmonids preexposed to sublethal concentrations of copper or zinc are able to survive exposure to concentrations of the same metal that would otherwise be lethal. Whether a combination of copper, zinc, or cadmium produces the same results remains to be shown. However, the lability of the lethal response with history of metal exposure poses great difficulties with regard to its use in hazard assessment. The question remains as to whether the most contaminated area of Buttle Lake is marginally toxic and the resident rainbow trout represent a proportion of survivors, or whether they are suffering from sublethal toxicity. A measure that can be related to toxicity would provide a method for the evaluation of toxic risk to a certain population of fish.

Our data show that the levels of metals in the livers of exposed fish are a poor measure because they are subject to extreme variation within one sample site. Furthermore, zinc, which is the predominant metal in the water, did not differ in the livers of fish from the most contaminated lake to the least.

When total levels of metals are measured in tissues, one cannot distinguish between the quantity which causes a biological response in the fish and that which is nonspecifically bound and without effect. It has been proposed that it is the binding of metals to high molecular weight proteins that is responsible for the toxicity of metals and that the binding of metals to low molecular weight metallothionein provides a detoxifying effect. This may be so, but the observations in Fig. 3A, B, and C show that there are marked differences in the distribution of zinc from that of the other two metals. Despite the much higher concentrations of zinc than of copper in the water to which the fish had been exposed, copper and a small amount of cadmium, but not zinc, were bound to metallothionein. In the high molecular weight fraction, zinc was found to exceed by far its concentration in the metallothionein fraction. It is known (Kägi and Nordberg 1979) that copper is much more tightly bound to metallothionein than cadmium, and cadmium is more tightly bound than zinc. Our findings are in accord with predictions based on these facts. In the presence of all three metals, as shown by our data, there would apear to be no detoxification by metallothionein synthesis against zinc. Significant quantities of copper were bound to metallothionein so that it is possible that this process did provide some protection to the fish against the toxicity of copper. On the other hand, it is to be noted that, for copper, the concentration in the high molecular weight fraction (Fig. 3A) increased in parallel with the increase in metallothionein levels. There is thus no evidence in our data in support of the "spill-over" hypothesis (Winge et al. 1973; Brown and Parsons 1978), i.e. that the concentration of metal in the high molecular weight proteins is kept down until the capacity of the animal to synthesize metallothionein is exceeded.

Whether the synthesis of metallothionein plays a role in detoxification of metals or not, it is evident from the data of Table 3 that its concentration in the livers of the fish exhibited

a downward trend from the most contaminated lake (South Buttle) to the least (Upper Quinsam). The other parameters that showed a similar trend were (a) zinc in the water, (b) cadmium and copper in the high molecular weight protein fraction, and (c) copper in the low molecular weight fraction.

As noted above, a determination of the metals at sublethal concentration in the water tells nothing about the state of the fish. Although the concentrations of metals in the high molecular weight proteins might be related to toxic manifestations, such a relationship remains to be shown.

In comparison, the levels of hepatic metallothionein are a specific measure of the biological response of the fish to metals, and may be measured using individual livers, specifically, sensitively and rapidly.

Acknowledgment

This work was funded in part by a Strategic Grant (NSERC G0246) from the Natural Sciences and Engineering Research Council of Canada, and by a contract with the Waste Management Branch, Ministry of Environment, Province of British Columbia. The cooperation of Dr R. Nordin, Mr C. McKean, and personnel of the Aquatic Studies Branch of the Ministry of the Environment is gratefully acknowledged.

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Factors Controlling Phytoplankton Production in Lake Vanda (77°S)

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VINCENT, W. F., AND C. L. VINCENT. 1982. Factors controlling phytoplankton production in Lake Vanda (77°S). Can. J. Fish. Aquat. Sci. 39: 1602–1609.

At least three floristically distinct communities of algae were distributed down the permanently ice-capped water column of Lake Vanda. Phytoplankton at each depth were highly specialized towards specific conditions of light and temperature. Maximum photosynthesis and algal biomass was at the bottom of the euphotic zone (55–57.5 m) immediately above a region of nutrient-rich anoxic water. This *Phormidium*-dominated community was adapted towards warm temperatures (18–20°C) but a dim light regime. Dissolved inorganic N to P ratios in this region were extreme (>5000:1) and the plankton demonstrated strong cellular P deficiency. A much smaller photosynthetic maximum was recorded in the middle of a large thermohaline convection cell at 25–30 m where P deficiency was less severe, but detectable. A microflagellate community immediately under the ice demonstrated the least response to phosphorus enrichment, but photosynthetic characteristics appeared to be more attuned to the low irradiances which prevail early in the growing season rather than to the relatively bright light regime during midsummer sampling. Algal biomass levels at most depths declined over 4 wk of sampling, further indicating that population maxima are timed early in the season. The low input of phosphorus relative to nitrogen exerts an overall control on phytoplankton biomass in Lake Vanda, but low production rates per unit biomass characterize the shade-adapted populations at all depths.

Key words: phytoplankton, photosynthesis, algal biomass, N to P ratios, ice cap

VINCENT, W. F., AND C. L. VINCENT. 1982. Factors controlling phytoplankton production in Lake Vanda (77°S). Can. J. Fish. Aquat. Sci. 39: 1602–1609.

Au moins trois communautés algales, de composition distincte, sont réparties dans la colonne d'eau recouverte de glace en permanence du lac Vanda. Le phytoplancton à chaque profondeur est très spécialisé par rapport à des conditions spécifiques de lumière et de température. La photosynthèse et la biomasse algale sont à leur maximum au fond de la zone euphotique (55–57,5 m), immédiatement au-dessus d'une région d'eau anoxique riche en nutriments. Cette communauté dominée par *Phormidium* est adaptée à des températures élevées (18–20°C) mais à une faible intensité lumineuse. Dans cette région, les rapports N:P inorganiques dissous sont extrêmes (>5 000:1), et le plancton accuse une importante déficience de P cellulaire. Un maximum photosynthétique beaucoup moindre a été enregistré au milieu d'une grande cellule de convection dans la thermocline à 25–30 m où la déficience en P, bien que détectable, est moins sérieuse. Une communauté de microflagellés juste au-dessous de la glace a la plus faible réaction à un enrichissement en phosphore, mais les caractéristiques photosynthétiques semblent plus sensibles aux faibles irradiances du début de la saison de croissance plutôt qu'à l'intensité lumineuse relativement élevée de la mi-été. Les niveaux de biomasse algale à la plupart des profondeurs ont baissé pendant les 4 sem de l'échantillonnage, autre signe que les maxima de population se produisent tôt dans la saison. Les faibles apports de phosphore par rapport à l'azote règlent l'ensemble de la biomasse phytoplanctonique dans le lac Vanda, mais de faibles taux de production par unité de biomasse caractérisent les populations adaptées à l'obscurité à toutes les profondeurs.

Received January 20, 1982
Accepted July 26, 1982

Reçu le 20 janvier 1982
Accepté le 26 juillet 1982

ALMOST 20 years ago Lake Vanda in the Antarctic Dry Valley region was identified as one of the world's clearest and most oligotrophic waters (Goldman et al. 1967). Since that time this permanently ice-covered lake has been the focus of considerable geochemical and geophysical debate (e.g. Hoare 1968; Bydder and Holdsworth 1977; Yoshida et al. 1975) but its biology has remained relatively unexplored. The early investigators on Lake Vanda reported a simple phytoplankton community of low standing crop and productivity, perhaps ultimately limited by temperature and the availability of nitro-

Printed in Canada (J6791)
Imprimé au Canada (J6791)

gen; addition of nitrate stimulated photosynthetic CO_2 fixation by both littoral and pelagic samples, and maximum photosynthesis was recorded in the warm, solar-heated, bottom waters of the lake (Goldman et al. 1967).

Subsequent studies on algae in culture and on other meromictic lakes of the Dry Valleys suggest that the controlling effects of temperature and nitrogen require further evaluation. For many species of phytoplankton the ^{14}C-photosynthesis bioassay appears to be an unreliable and sometimes misleading guide to N or P limitation (Healey 1979). Comparisons between several lakes of South Victoria Land indicate that the low light regime beneath their permanent ice caps may exert a far stronger controlling influence on production per unit biomass than the direct effects of low temperature (Vincent 1981b). The current study was therefore designed to reassess the relative importance of light and temperature, and nitrogen and phosphorus supply for phytoplankton production down the stratified water column of Lake Vanda. We present the first chlorophyll *a* data for Vanda and detail the distribution of algal species with respect to depth over the field season. We then examine the physiological response of phytoplankton from discrete strata to a range of physical and chemical conditions.

Study Site

Lake Vanda (77°35′S, 161°40′E) lies 47 km from the coast, in the Wright Valley, South Victoria Land, at an altitude of 143 m. The lake is 8.5 km long, 2.4 km wide, with a maximum depth relative to the upper ice surface of 68 m. The unusual temperature and salinity characteristics of Lake Vanda were first reported by Armitage and House (1962) and have been the subject of detailed surveys subsequently. Temperatures rise with increasing depth to a maximum of 23.5°C at the bottom of the lake where the conductivity is three times that of seawater. The lake contains several isothermal isohaline layers that are isolated from each other and from the ice surface and lake floor by layers of density-stratified water. Each homogeneous layer is 3 m or less in thickness, with the exception of the thickest band which extends between 17 and 37 m in depth. Within each of these bands strong thermohaline convection currents maintain homogeneous temperatures and salinities (Hoare 1966, 1968; Ragotzkie and Likens 1964).

Lake Vanda is fed by the Onyx River which carries midsummer meltwater 40 km inland from coastal piedmont glaciers. The river flows continuously for 1−2 mo each year with a discharge typically around 1 $m^3 \cdot s^{-1}$, but with peaks up to 9 $m^3 \cdot s^{-1}$ (Chinn 1980).

Methods

Sampling, measurements, and incubations were performed through 10-cm-diameter holes bored through the permanent ice cap. Samples were removed with a 1-L discrete-depth water sampler. Photosynthetically available radiation (PAR) was measured with a Lambda submersible quantum probe fitted with various color filters (blue: Hoya 80B; green: Hoya BX1; red: Vivitar 25A).

TABLE 1. Light extinction properties of Lake Vanda, December 17, 1980. The ice was 330 cm thick at the sampling site. Extinction coefficients for the water are for the region from the lower ice surface to 45 m.

	Extinction coefficient (m^{-1})		% surface irradiance	
Waveband	Ice	Water	3.25 m	57.5 m
White	0.59	0.05	14.7	0.7
Red	0.95	0.46	4.5	<0.01
Green	0.50	0.06	19.7	0.6
Blue	0.42	0.04	25.6	1.8

Samples for chlorophyll analysis were filtered through glass fiber filters (grade GF/C) which were stored frozen until extraction by boiling methanol. The extracts were cleared by centrifugation and measured by fluorometry (Holm-Hansen and Riemann 1978). Estimates of chlorophyll *a* were not corrected for degradation products (Talling and Driver 1963).

In vivo fluorescence of chlorophyll *a* was measured with a Turner III fluorometer, with (F_b) and without (F_a) DCMU as described by Vincent (1980). Cellular fluorescence capacity (CFC) values were calculated ($1 - F_a/F_b$) as an index of photochemical capacity per unit chlorophyll (Vincent 1980).

Phytoplankton samples were preserved immediately after collection with Lugol's iodine or 2% (final concentration) glutaraldehyde. The cells were later sedimented and counted by inverted microscopy (Utermöhl 1958).

Photosynthetic rates were measured by in situ incubations in duplicate 60-mL light and dark bottles with [^{14}C]HCO_3 [35 μCi (1 Ci = 37 GBq) per bottle]. At the end of each 24-h incubation the labeled plankton were filtered onto 0.22-μm Millipore filters. These were acid-fumed for 24 h to remove ^{14}C-carbonates, air-dried, and counted by liquid scintillation spectrometry. Samples for dissolved inorganic carbon analysis were injected into sealed 30-mL Hypovials and immediately preserved with glutaraldehyde (2% v/v final concentration). The carbon levels were later measured by infrared CO_2 analysis.

Samples for nutrient analysis were filtered through acid-washed glass fiber filters (GF/C) immediately after collection and were stored frozen. All analyses were on a Technicon AutoAnalyzer II as described by Vincent et al. (1981). Total dissolved nitrogen and phosphorus concentrations were measured after ultraviolet light conversion to nitrate and phosphate. Particulate nitrogen and phosphorus were filtered onto acid-washed GF/C filters and stored frozen. This material was subsequently Kjeldahl-digested and then analyzed for phosphate and ammonium.

Three assays for nutrient deficiency were applied to the Lake Vanda plankton. Nitrogen deficiency was tested by the ammonium enhancement assay of Yentsch et al. (1977). When algal cells deficient in nitrogen are enriched with ammonia, they rapidly accelerate their rate of dark CO_2 fixation. Lake Vanda samples were therefore incubated at the depth of collection in dark bottles for 5 h with and without NH_4-N (50 $mg \cdot m^{-3}$ final concentration). Dark carbon uptake was measured with [^{14}C]HCO_3 as above. As a further guide to both nitrogen and phosphorus demand, lake water samples were incubated for 6 h in situ with an enrichment of nitrogen

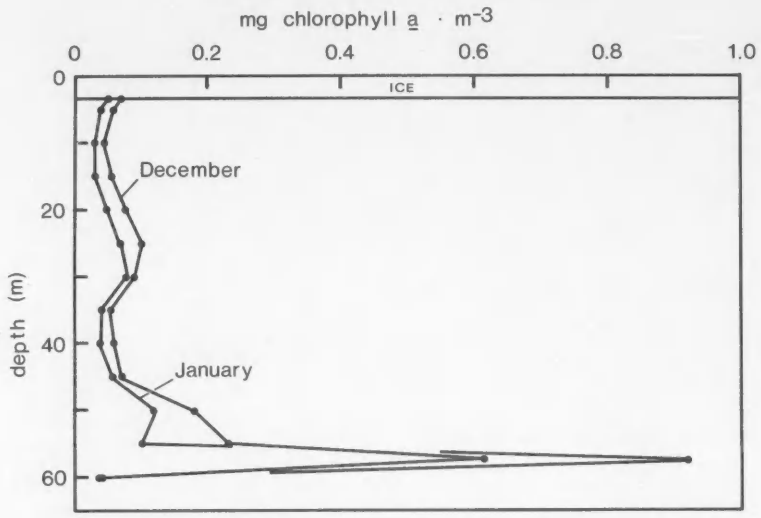

FIG. 1. Distribution of chlorophyll *a* with depth December 13, 1980 and January 11, 1981.

TABLE 2. Photosynthesis in Lake Vanda, December 21–22, 1980. Irradiance values were calculated as the average intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$) over the 24-h in situ incubation.

Depth (m)	Photosynthesis[a]	Assimilation number[b]	Photosynthetic efficiency[c]
3.25	130	108	2.6
5	82	83	2.1
15	160	215	6.0
25	223	131	8.0
30	189	104	8.9
40	182	161	17.1
50	463	188	18.2
55	271	90	10.7
57.5	1664	138	27.2
60	Nil		

[a]Micrograms C fixed $\cdot m^{-3} \cdot d^{-1}$.
[b]Micrograms C fixed $\cdot mg^{-1}$ chl*a* $\cdot h^{-1}$.
[c]Micrograms C fixed $\cdot mg^{-1}$ chl*a* $\cdot$ unit irradiance$^{-1} \cdot h^{-1}$.

(50 mg NH_4-N $\cdot m^{-3}$) and phosphorus (5 mg PO_4-P $\cdot m^{-3}$). Seston N to P ratios were compared before and after the enrichment (Vincent 1981a). Finally, samples were incubated in situ for 4–6 d in 60-mL light bottles with N, P, N plus P, or no enrichment (levels as above). Photochemical capacity was measured by the CFC index before and after the incubation.

Results

Extinction coefficients for total PAR and blue and green wavebands in the surface waters of the lake approximated the low values obtained by Goldman et al. (1967) almost 20 yr previously (Table 1). The most penetrating waveband measured was blue light; red light was strongly absorbed by the ice and rapidly extinguished with depth.

Chlorophyll levels (Fig. 1) were very low throughout the water column but rose to a sharp maximum at 57.5 m, just above the anoxic zone (transition from oxic to anoxic water was at 59–60 m). Two less-pronounced maxima were recorded higher in the water column — one immediately beneath the ice, and another between 15 and 35 m, in the center of the large thermohaline convection cell. Between December 13 and January 11, there was a decline in chlorophyll levels at all depths, except at 57.5 m where higher values were recorded in January. In view of the steep chlorophyll gradient at the bottom of the oxygenated water column, this increase may reflect sampling error rather than a real increment in biomass between the two dates. A difference in sampling depth of only a few centimetres could have resulted in a large difference in chlorophyll *a* levels, even on the same day.

In situ photosynthetic rates (Table 2) closely followed the distribution of chlorophyll, with a sharply defined maximum at 57.5 m. Lesser peaks occurred immediately under the ice and at 25–30 m. Assimilation numbers (photosynthesis per unit chlorophyll) demonstrated no marked variation, but light-capturing ability per unit chlorophyll (photosynthetic efficiency) increased with increasing depth to a maximum value just above the oxycline.

Further samples were incubated for 24 h with [^{14}C]HCO_3 on December 27–28, at 55, 57.5, 60, 62.5, 65, and 67.5 m, with and without DCMU, to test for bacterial photosynthesis. No significant differences between light and dark bottle incubations were recorded in anoxic water below 60 m. Photosynthesis at 55 and 57.5 m was completely eliminated by the DCMU treatment.

Plankton samples from three strata were each incubated with [^{14}C]HCO_3 at six depths in the lake to determine their relative photosynthetic response to light and temperature. Each assemblage demonstrated maximum rates of photo-

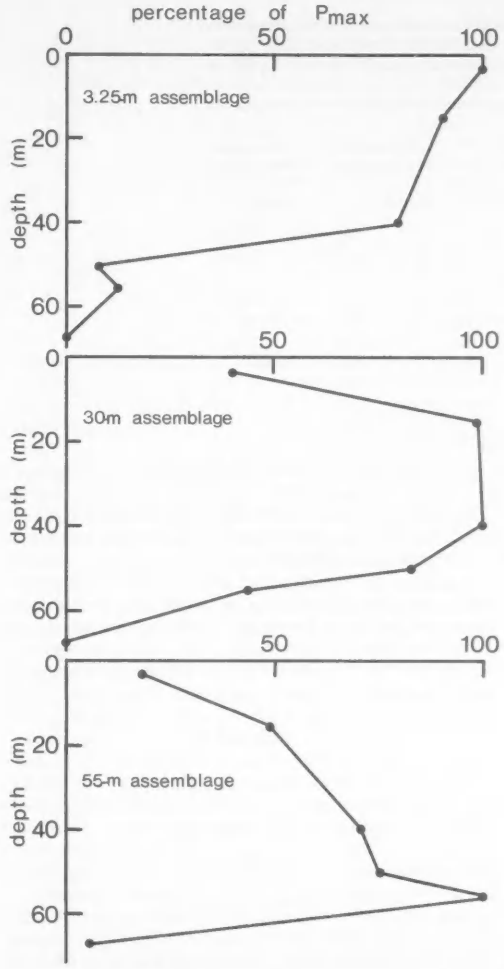

FIG. 2. Effect of displacement on algal photosynthesis December 23–24, 1980. Duplicate light and dark bottles were incubated 24 h at each depth. Rates are expressed as a percentage of the maximum recorded for each assemblage.

synthesis at or close to its depth of collection (Fig. 2) indicating a high degree of specialization towards specific physical conditions. The magnitude and type (positive or negative) of response to displacement were different for each assemblage. Two sets of displacement depths were particularly informative. Between 15 and 40 m (either side of the large convection cell), the temperature variation was almost negligible (6.5 vs. 7.5°C) but light availability changed 3.5-fold, from 8.1 to 2.3% of surface PAR. The 3.25-m assemblage responded to this light decrease by only an 11% drop in photosynthesis. By contrast, the 55-m community was strongly inhibited by the higher light level. The 30-m assemblage

TABLE 3. Cell volumes of phytoplankton dominants in three depth regions of Lake Vanda (— = not detectable).

	Depth (m)	Cell volume (10^6 $\mu m^3 \cdot L^{-1}$) Dec. 16	Jan. 11
Ochromonas miniscula	3.25	41.5	33.8
	30	14.5	8.7
	55	—	—
Polytomella sp.	3.25	19.6	0.9
	30	—	—
	55	—	—
Chlorella sp.	3.25	2.5	2.0
	30	3.4	1.5
	55	—	—
Chlamydomonas globosa	3.25	—	—
	30	3.0	1.4
	55	1.9	—
Phormidium fragile	3.25	—	—
	30	76.6	65.1
	55	13.4	13.0
P. antarcticum	3.25	—	—
	30	71.2	38.8
	55	98.5	4.2
P. frigidum	3.25	—	—
	30	—	—
	55	17.6	15.2
Synechocystis (?)	3.25	—	—
	30	—	—
	55	2.9	5.3

TABLE 4. Nitrate–nitrogen and soluble reactive phosphorus (SRP) levels in the Onyx River, December 31, 1980. Each value is the mean for duplicate samples (± range) collected 5 min apart.

Site	Distance upstream from Lake Vanda, km	NO_3–N ($mg \cdot m^{-3}$)	SRP ($mg \cdot m^{-3}$)
1	6	51.5±2.0	3.2±1.1
2	4	35.8±0.9	1.7±0.1
3	2	28.6±0.6	0.6±0.1

demonstrated no difference in response to incubation at the two depths suggesting a wide adjustment to this range of intensities. A second useful comparison was between the incubations at 50 and 55 m. Over this 5-m-depth interval, light availability changed very little (1.2–0.9% of surface PAR) but temperatures increased by 6°C (12.5–18.5°C). Photosynthesis by the 55-m sample dropped by 25% when displaced from its depth of collection to 50 m. A reverse response was demonstrated by the 30-m assemblage for which photosynthetic rates were 89% higher at 50 m relative to 55 m. Photosynthesis by the 3.25-m community was very low (<15% of optimum) at both 50 and 55 m, and there was no significant difference between the two incubations.

Phytoplankton samples from strata containing the three assemblages tested by displacement were examined by Utermöhl microscopy. The three communities differed markedly in species composition (Table 3). Microflagellates were conspicuous components of the two upper assemblages, but at the

TABLE 5. Seston and dissolved inorganic N to P ratios (wt/wt), and ammonium enhancement response. F values are for the difference between dark CO_2 fixation with and without ammonium additions. Values less than 2.92 are not significant at $P > 0.1$ (df = 2) (— = not tested).

Depth (m)	Seston Nitrogen ($mg \cdot m^{-3}$)	Seston Phosphorus ($mg \cdot m^{-3}$)	Seston N/P	Dissolved inorganic N/P[a]	Ammonium enhancement (F values)
3.25	10.2	0.8	12.8	70	0.16
15	3.7	0.2	18.5	39	1.21
25	9.8	0.6	16.3	104	0.87
35	7.0	0.2	35.0	84	—
45	5.7	0.3	19.0	103	0.83
50	7.8	0.4	19.5	4978	—
55	10.5	0.2	52.5	>52 912	<0.01
57.5	17.5	0.4	43.7	5982	—
60	26.2	0.2	131.0	2100	—

[a]Inorganic N/P = (NO_3−N plus NO_2−N plus NH_4−N)/(soluble reactive P). Complete data set for inorganic nutrients presented in Vincent et al. 1981.

bottom of the euphotic zone blue-green algal populations dominated.

The single inflow to Lake Vanda, the Onyx River, was sampled at its point of entry to the lake within the period of peak flow (late December). The total dissolved nitrogen level was 178 $mg \cdot m^{-3}$ (range for duplicate samples of ±16) and total dissolved phosphorus was 2.9 ± 0.1. This high ratio of N to P load (61.5:1) suggested that phosphorus might be in relatively short supply for phytoplankton growth in the lake. A comparison of nitrate and soluble reactive phosphorus (SRP) levels further up the river demonstrated a similarly high ratio of N to P with some evidence of nutrient stripping by stream biota before discharge to the lake (Table 4). Between sites 1 and 3 there was no change in discharge but the nitrogen and phosphorus fell by 8.8:1, which closely approximates the ratio found in algal tissue.

Dissolved inorganic N to P ratios in the surface waters of Lake Vanda (Table 5) reflected those within the Onyx River. At the bottom of the euphotic zone the ratio increased to extreme values dictated primarily by an accumulation of nitrate produced by nitrifier activity (details in Vincent et al. 1981). Particulate levels of phosphorus were very low throughout the water column. The ratio of nitrogen to phosphorus in the seston similarly increased with increasing depth to very high values at the bottom of the euphotic zone.

No stimulation of dark CO_2 fixation by addition of ammonia was recorded for any depth assayed (Table 5). Seston N to P ratios dropped in all samples incubated with a nitrogen plus phosphorus enrichment, but the decrease was statistically significant for only the 30-m assemblage (Table 6). This response contrasted with a sample tested at the same time from Lake Fryxell, a more productive lake in the adjacent valley. The seston from just under the ice in Fryxell selectively accumulated nitrogen from a N plus P enrichment, and the seston N to P ratio significantly increased.

The photochemical response to nutrient enrichment was measured by CFC assays on further plankton samples from three strata. After 2 d the 55-m samples demonstrated significantly increased CFC values in response to phosphorus enrichment, but in those samples incubated with nitrogen, CFC was reduced relative to the control (Table 7). There was a significant decrease in the control (no nutrients) over this period indicating an adverse effect of containerization on this bottom euphotic assemblage. At higher depths in the water column there were no significant changes in CFC over the first 2 d and the in situ incubations were therefore extended for 2 more days. By day 4 there was a positive response to phosphorus by the 30-m, but not the 3.25-m assemblages. In both sets of samples enriched with nitrogen, there was a significant decline in CFC. The final CFC controls for both depths did not change over the course of the incubation.

Discussion

Nitrogen has been identified as the primary nutrient limiting algal production in several Antarctic lakes. However, the supporting evidence for these observations has generally been sparse and derived from a restricted array of measurements and methodologies. The photosynthetic CO_2 fixation response to nitrate enrichment reported for Vanda (Goldman et al. 1967) has been recorded in Lake Skallen Oike (69°40′S, 39°24′E) where SRP levels were relatively high (Tominaga 1977). Hand and Burton (1981) reported undetectable levels of dissolved inorganic nitrogen, but abundant phosphorus in Ace Lake in the Vestfold Hills (68°28′S, 78°11′E). From this they concluded that algal production may depend upon the rate of supply of inorganic nitrogen by meltwater to the lake each summer. In Lake Fryxell, in the adjacent valley to Lake Vanda, water samples from immediately under the ice contained low levels of inorganic N, and the plankton responded positively to ammonium enrichments by enhanced dark CO_2 fixation and increased photochemical capacity as meaured by CFC (Vincent 1981b). Selective accumulation of nitrogen by the Fryxell plankton from an N plus P enrichment reported in the present study provides supportive evidence for a persistent shortage of nitrogen relative to phosphorus for algal growth in that lake.

We could find no evidence of nitrogen limitation in Lake Vanda. Nitrogen was in abundant supply relative to phosphorus, both within the meltwater inflow and within all strata

TABLE 6. Seston N to P ratios before and after 6 h enrichment with 50 mg $NH_4-N \cdot m^{-3}$ plus 5 mg $PO_4-P \cdot m^{-3}$. Each value is for duplicate samples ± range. *t* values test the significance of change for each lake water.

	Lake Vanda			Lake Fryxell
	3.25 m	30 m	55 m	4.5 m
Seston N/P (initial)	19.3±2.2	20.2±0.2	48.4±7.7	10.6±0.2
Seston N/P (final)	17.6±2.6	17.6±0.3	23.6±5.7	15.2±1.5
t-value	0.5	7.2	2.6	3.5
probability	>0.1	<0.025	>0.1	<0.1

beneath the ice. Dissolved inorganic N to P ratios were well in excess of 15 to 1 at all depths. No ammonium enhancement of dark CO_2 fixation was recorded in any sample tested. Photochemical capacities of the three separate assemblages (3.25, 30, 55 m) were not stimulated by enrichment with ammonium, but in fact for two of the communities tested there was a significant reduction in CFC relative to the control during the plus N incubation.

Several lines of evidence favor phosphorus as the primary nutrient limiting algal growth in Lake Vanda. SRP concentrations were very low in the Onyx River and appeared to be further reduced by biological stripping before discharge to the ultra-oligotrophic surface waters of the lake. At all depths between the lower ice surface and 55-m lake water, SRP values were less than 1 $mg \cdot m^{-3}$ (Vincent et al. 1981). Particulate phosphorus levels were similarly low, and the seston N to P ratio at most euphotic depths was in excess of the typical range found in algae growing under nutrient sufficient conditions. This ratio dropped upon N plus P enrichment as phosphorus but not nitrogen was accumulated by the plankton, thereby demonstrating a higher demand for P relative to N. Both the 30- and 55-m samples significantly increased their photochemical capacity during enrichment with P.

Both rapid physiological and longer-term bioassays for nutrient demand and deficiency must be interpreted with caution. The former are highly sensitive to recent light and nutrient history, and physiological transients; the latter are prone to artifacts of containerization (Vincent 1981a). However, the assay responses reported here from a variety of time scales (hours to days) consistently indicate a shortage of phosphorus relative to nitrogen for plankton primary production. This finding is strongly supported by the direct analyses of particulate material and dissolved nutrients.

Floristically distinct communities of phytoplankton occupied different depth regions of Lake Vanda where they were highly specialized towards the specific conditions of light and temperature, and probably nutrient supply and salinity, within each layer. Like other lakes of the region (Vincent 1981b), maximum biomass and photosynthesis occurred just above the interface between oxic water and the nutrient-rich anoxic zone. The blue-green algae which dominated this community did not appear to be photosynthetically active in the deeper, anaerobic layers, as they are in some meromictic lakes (e.g. Solar Lake, Cohen et al. 1977). In the region of maximum chlorophyll, SRP levels were higher than in the rest of the euphotic zone (from 0.6 $mg \cdot m^{-3}$ at 56 m to 3.1 $mg \cdot m^{-3}$ at 59 m). Temperatures were warm (20°C) by comparison with higher depths and more typical of temperate lakes in summer. However, the stimulatory effects of temperature were probably offset by low light availability (<1% of surface irradiance), and productivity per unit biomass was as low in the deep euphotic zone as it was in the cooler waters immediately beneath the ice.

Deep chlorophyll maxima are a ubiquitous feature of thermally stratified euphotic zones (Richerson et al. 1978) and are often particularly striking in the highly stable waters of meromictic lakes (e.g. Cohen et al. 1977). In the Canadian Experimental Lakes Area (ELA) metalimnetic algal maxima have been attributed to slow rates of loss (respiration, grazing, sedimentation) rather than elevated rates of production (Fee 1976). Like other permanently ice-capped lakes of the Dry Valleys, Lake Vanda contains no crustaceous zooplankton (Goldman et al. 1967; W. F. Vincent personal observation) and grazing losses cannot be considered a determinant of algal distribution. The deep production zone of Vanda is uniquely a region of minimal energy input yet highest euphotic temperatures under which respiratory losses may be maximal. Sedimentation may also be more rapid than in the thermohaline convection cells higher in the water column. In marked contrast to ELA maxima, the deep biomass peak in Lake Vanda must be considered a community of elevated gross production rates, favored by the relative abundance of nutrients across the oxycline, with loss rates that are probably higher than in other euphotic strata.

A community of microflagellates and *Phormidium* dominated the middle euphotic zone where the phytoplankton appear to be retained and concentrated in the center (25−30 m) of the large thermohaline convection cell. In this region both chlorophyll *a* and photosynthetic rates were higher than in the overlying water despite lower irradiances. The center of this convective layer may resemble Stommel's (1949) "zone of retention" in Langmuir circulation cells where algal sedimentation is greatly reduced by horizontal and vertical water movements. Phosphorus deficiency in this region was less severe than in the deep euphotic zone, but seston N to P ratios were high and responded to P enrichment.

Immediately beneath the ice, temperatures rose from 0 to 4.5°C within several centimetres deep. This density gradient may aid the microflagellates which dominate the upper euphotic community in maintaining their position. Phosphorus

TABLE 7. Photochemical response to enrichment, as measured by DCMU-induced fluorescence of chlorophyll (CFC index). Each value is the mean for duplicate lake water samples incubated for 2 d (55 m) or 4 d (3.25 and 30 m) at the depth of collection with 50 mg·NH_4−N·m^{-3}, 5 mg PO_4−P·m^{-3}, or no addition (control). Numbers in parentheses are F values for orthogonal comparisons between the control and treatment CFC. F values greater than 7.7 are significant at the $P < 0.05$ level (df = 1, 4).

Depth (m)	CFC units			
	Initial	Control	Plus N	Plus P
3.25	0.271	0.272	0.179	0.262
	(<0.01)		(11.08)	(<0.01)
30	0.192	0.200	0.077	0.334
	(0.04)		(18.96)	(25.30)
55	0.358	0.214	0.163	0.547
	(10.62)		(1.31)	(56.95)

availability may be slightly higher in this region as a result of freeze concentration of salts as the ice cap thickens over winter, and the inflow of Onyx River water under the ice during summer. In this region of the water column, seston N to P ratios were lowest and there was no clear evidence of strong cellular P deficiency.

The ice cap covering Lake Vanda is relatively thin and highly transparent compared to other Dry Valley lakes, e.g. in Lake Fryxell 1% of surface irradiance penetrates the ice (Vincent 1981b), cf. 15% in Vanda (Table 1). Light-capturing efficiency was understandably less in Lake Vanda relative to Fryxell; however, the Vanda populations do appear to be more shade-adapted than ambient light levels would suggest. Immediately under the ice the average irradiance over 24 h in early January was 130 $\mu E \cdot m^{-2} \cdot ^{-1}$. This lies within the range generally considered optimal for algal photosynthesis (e.g. Harris 1978), yet photosynthetic rates per unit chlorophyll in Vanda were still very low at these upper euphotic depths (ca. 0.1 mg $C \cdot mg^{-1}$ chl$a \cdot h^{-1}$) by comparison with those rates typical of temperate latitudes (1−10 mg $C \cdot mg^{-1}$ chl$a \cdot h^{-1}$). From 3.25 m to 40 m there was an 84% decrease in light intensity (temperature rose only 3°C), yet displacement of the phytoplankton from the upper to lower depth resulted in only a 20% drop in photosynthetic rates. These observations suggest that the sub-ice community was adapted to lower light levels than those measured during the period of sampling.

Between mid-December and mid-January there was a marked decline in chlorophyll *a* levels and in cell biomass of all but one of the phytoplankton dominants. This is consistent with previous observations on Lake Fryxell (Vincent 1981b): phytoplankton maxima in these lakes are probably timed very early in the season after nutrients have accumulated to a maximum over winter (mineralization by bacteria, diffusion from the anoxic zone, freeze-concentration at the ice−water interface) and when ambient irradiances have just begun to rise above the compensation point for algal photosynthesis. The most successful phytoplankton species in these lakes may therefore be shade-adapted forms capable of efficiently capturing the low irradiances in spring to take up and store a very limited supply of nutrients. The shade characteristics of Vanda sub-ice populations, living under relatively bright light conditions in midsummer, is supportive evidence of an early season phytoplankton maximum. During the brief 2−3 mo that this portion of Antarctica is accessible to limnologists, the primary production of Dry Valley lakes has passed its peak, and the planktonic algae are in decline. These populations demonstrate a physiology more attuned to irradiance conditions prevailing during early season growth than to the light regime experienced over the period of midsummer sampling. Thus low light appears to have a more controlling effect on productivity per unit biomass than temperature, but in Lake Vanda phosphorus availability exerts an overall control on production by limiting algal biomass.

Phytoplankton concentrations in Lake Vanda are comparable with ultra-oligotrophic waters in the Canadian Arctic. In Char Lake (74°42′N, 94°50′W), chlorophyll *a* values fall within the range 0.1−0.7 mg·m^{-3} (Kalff and Welch 1974); in Lake Vanda upper euphotic concentrations were typically less than 0.1 mg chlorophyll $a \cdot m^{-3}$, but rose to 0.7−0.9 mg·m^{-3} in the region of the deep production peak. In both Vanda (this study) and Char Lake (Rigler 1978), phosphorus appears to exert a stronger constraint on algal biomass than nitrogen; however, the controls on production per unit biomass (P/B) appear to differ markedly between the two systems. The cold temperatures of Char Lake depress P/B to a maximum assimilation number of 225 $\mu g\ C \cdot mg^{-1}$ chlorophyll $a \cdot h^{-1}$ (Kalff and Welch 1974). In Lake Vanda, temperatures attain higher values more typical of temperate lakes, yet assimilation numbers are all lower than in this northern counterpart. In Lake Vanda, and probably other ice-capped lakes of Antarctica, rates of community metabolism per unit biomass appear to be limited by low energy input to the euphotic zone, rather than by low temperatures more typical of Arctic waters studied to date.

Acknowledgments

We thank Ms K. Law for technical assistance, Antarctic Division DSIR, and the U.S. Navy for logistic support in the field; Drs T. Torii, S. Nakaya, W. Green, and D. Canfield for useful discussions; Drs E. White, M. Timperley, and G. Coulter for critically reviewing the manuscript; and Ms J. Simmiss for typing each draft. Fieldwork was performed during the 1980−81 New Zealand Antarctic Research Programme.

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Stock Definition in Atlantic Herring (*Clupea harengus harengus*): Genetic Evidence for Discrete Fall and Spring Spawning Populations

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KORNFIELD, I., B. D. SIDELL, AND P. S. GAGNON. 1982. Stock definition in Atlantic herring (*Clupea harengus harengus*): genetic evidence for discrete fall and spring spawning populations. Can. J. Fish. Aquat. Sci. 39: 1610–1621.

Ripe Atlantic herring (*Clupea harengus harengus*) were sampled from seven discrete spawning grounds in the Gulf of Maine and Gulf of St. Lawrence over a period of 3 yr. Genetic polymorphisms were observed at 13 enzyme loci by starch gel electrophoresis. Five highly polymorphic loci were used to assess population structure of herring stocks by contingency analysis of log-likelihood differences in gene frequencies. Significant temporal variation was observed at several localities. Within both spring spawning and fall spawning populations, significant spatial heterogeneity was noted for particular years, but was not temporally stable. By contrast, overall heterogeneity between spring and fall spawning populations was highly significant indicating genetic isolation of spring spawning populations in the Gulf of St. Lawrence from fall spawning aggregates in the Gulf of St. Lawrence and Gulf of Maine. The low levels of genetic heterogeneity and absence of both temporal stability within fall spawning samples and spatial stability among samples is not consistent with the existence of more than a single genetic population of fall spawning herring in the northwest Atlantic.

Key words: *Clupea harengus harengus*, population genetics, biochemical genetic variation, stock differentiation

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Nous avons prélevé des échantillons de harengs de l'Atlantique (*Clupea harengus harengus*) sur le point de frayer à sept frayères distinctes du golfe du Maine et du golfe du Saint-Laurent sur une période de 3 ans. L'électrophorèse sur gel d'amidon a permis d'observer un plymorphisme génétique à 13 loci d'enzyme. Nous en avons choisi cinq nettement polymorphes pour évaluer la structure de population de stocks de harengs par analyse de contingence des différences de probabilité dans la fréquence des gènes. On a trouvé une variation temporelle significative à plusieurs endroits. Dans des années particulières, nous avons noté une hétérogénéité spatiale significative tant dans les populations de reproducteurs de printemps que dans celles des reproducteurs d'automne. Cette hétérogénéité n'est toutefois pas stable dans le temps. Par contre, l'hétérogénéité entre reproducteurs de printemps et reproducteurs d'automne est très significative, signe d'une séparation génétique entre les populations de reproducteurs de printemps du golfe du Saint-Laurent et toutes les agglomérations reproductrices du golfe du Saint-Laurent et du golfe du Maine. La faible hétérogénéité génétique et l'absence, à la fois de stabilité temporelle dans les échantillons de reproducteurs d'automne et de stabilité spatiale entre échantillons, ne s'accordent pas avec l'existence de plus d'une population génétique de reproducteurs d'automne dans l'Atlantique nord-ouest.

Received August 12, 1981
Accepted August 23, 1982

Reçu le 12 août 1981
Accepté le 23 août 1982

ATLANTIC herring (*Clupea harengus harengus*) are a significant resource along the eastern coast of Canada and the United States. The species has been continuously exploited since precolonial times when prodigious populations were commonly observed in coastal communities (Bigelow and Schroeder 1953). More recent biomass fluctuations, possibly resulting from intense multinational fishing efforts, have created a need for accurate information on the population biology of this species. More specifically, information on the extent

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Printed in Canada (J6589)
Imprimé au Canada (J6589)

of reproductive isolation among spawning groups and the extent of population mixing during non-reproductive periods is essential for species management.

Major features of the biology and life cycle of Atlantic herring have been established (Blaxter and Holliday 1963), but detailed life history characteristics are poorly understood. In the western North Atlantic, reproduction is restricted to two intensive bouts, one in the fall (September–November) and one in the spring (April–May). At these times, large numbers of mature adults spawn at relatively discrete geographic locations (Boyar et al. 1973). Along the northeast coast of North America, spring spawning aggregations are currently restricted to localities in and around the Gulf of St. Lawrence, whereas fall spawning occurs in numerous areas, particularly the Gulf of Maine and the Gulf of St. Lawrence. Individual spawning aggregations have historically been considered by fisheries managers to be distinct stocks. Iles and Sinclair (1982), for example, have recently developed a mechanism to explain stock localization and abundance. However, the actual extent and duration of population mixing during various stages of the life cycle of herring is unknown and has been the point of repeated speculation (Anon. 1977; Vernberg 1977); fish tagged in the Gulf of Maine, Georges Bank, and Nova Scotia mix during non-spawning migratory periods (Spiers 1977; Stobo 1976). Although adults annually concentrate at specific locations to spawn, the fidelity of particular stocks to a specific spawning area is unknown. If, as has been widely assumed, fidelity of returning adult fish to spawning sites is essentially absolute, individual stocks would represent discrete reproductively isolated populations.

Central to this problem is the historical fisheries biology concept of a fish "stock" as an exploitable unit, which may or may not include members of a different genetic population (Larkin 1972; MacLean and Evans 1981). Yet, efficient exploitation of multiple stocks by a common fishery is based upon the assumption that stocks are reproductively isolated (Paulik et al. 1967), i.e. a stock is a genetic population. Thus, the problem of population definition and gene flow among herring must be therefore approached from a strictly genetic viewpoint. The only period in the life history of the herring where it is possible to examine reproductive isolation is during spawning. Because of mixing at other life history stages, geographic comparisons during non-spawning periods are of no value in delimiting populations (Williams et al. 1973). To define populations, it is critical that only spawning fishes be examined.

Fisheries scientists have attempted to use a variety of techniques to discriminate herring populations including internal and external morphology, biological tags, and protein variants. Meristic characters have been employed with varying results. Otoliths have been successfully used to demonstrate significant differences between spring and fall spawning populations (Côté et al. 1980). However, despite characterization of some stocks by these methods, variation in morphological characters may have a dominant environmental component. High degrees of morphometric similarity between spawning aggregations may result if environmental conditions influencing these characters are similar; conclusive evidence of biological isolation among populations requires demonstration of genetic differences (Messieh and Tibbo 1971).

For over 20 yr, biologists have studied biochemical variation in Atlantic herring in attempts to define population structure. Because it can provide genetic identification without confounding environmental influences, biochemical variation as assayed by electrophoresis is a powerful tool for population discrimination and identification. Previous studies have identified a portion of the biochemical genetic variation in Atlantic herring (Mairs and Sindermann 1960; Sindermann and Honey 1963; Odense et al. 1966; Wilkins and Iles 1966; Klose et al. 1968; Lush 1969; Naevdal 1969a, b; Simonarson and Watts 1969; Ridgway et al. 1970; Wolf et al. 1970; Engel et al. 1971; DeLigny 1972; Lewis and Ridgway 1972; Odense et al. 1973; Schmidtke et al. 1975; Odense 1980; Andersson et al. 1981). However, these studies are of limited value in assessing population structure because they did not meet two essential criteria: (1) the genetic basis (i.e. heritability) of observed variation must be adequately established (Fairbairn and Roff 1980), and (2) only sexually mature fish in spawning condition must be sampled from discrete spawning grounds.

This paper presents the results of an intensive study to characterize the genetic variation within and among geographically distinct samples of Atlantic herring. A large number of proteins were resolved from which 13 polymorphic loci were extensively examined in reproducing adults at specific spring and fall spawning localities over a 3-yr period. We feel that this study represents a rigorous examination of northwest Atlantic herring population structure because: (1) the Mendelian heritability of the polymorphic loci employed was established through breeding experiments (Kornfield et al. 1981); (2) only sexually competent fish from known spawning aggregates were sampled; and (3) the temporal stability of gene frequencies at all spawning/sampling sites was tested over two (in some cases, three) consecutive years.

For two reasons we emphasize a temporal, rather than primarily a spatial, analysis of our data. First, temporal stability of gene frequencies is one of the essential prerequisites for realistic use of electrophoretic variation in defining populations (Allendorf and Utter 1979; Utter et al. 1980). Inferences about underlying population structure deduced from particular patterns of spatial variation may be wholly inaccurate if such patterns vary markedly over time. Second, because significant differences have been demonstrated between spring and fall spawning herring populations in the Gulf of St. Lawrence using morphological characters (Côté et al. 1980; Messieh 1972, 1975; Messieh and Tibbo 1971), temporal analysis of electrophoretic data from these populations allows us to gauge the sensitivity of our genetic assays. That is, failure to discriminate electrophoretically between these populations would cause us to question our general ability to identify herring populations, should they exist in other regions.

Materials and Methods

Samples of Atlantic herring were collected in conjunction with commercial fisheries at seven spawning locations in the Gulf of Maine and Gulf of St. Lawrence (Table 1). Two locations were sampled only in 1979, three in 1979 and 1980, and two in 1978, 1979, and 1980. Collections were made during the peak of reproduction at discrete spawning

TABLE 1. Collection localities and estimates of genetic variability in samples of Atlantic herring.

Geographic location		Date collected		Average no. individuals per locus	Proportion of loci	
					Polymorphic per population[a]	Heterozygous per individual
		Spring spawning localities				
Carleton, Que.	48°10′N; 66°00′W	May 14, 1979	C79	43.9	0.143	0.044
		May 6, 1980	C80	78.5	0.095	0.046
Ile Verte, Que.	48°00′N; 69°30′W	May 13, 1979	IV79	66.5	0.095	0.048
		May 5, 1980	IV80	98.0	0.095	0.044
		Fall spawning localities				
Miscou, Que.	48°10′N; 64°20′W	Sept. 22, 1979	M79	47.0	0.119	0.059
Stonehaven, N.B.	47°50′N; 65°20′W	Sept. 22, 1979	S79	40.8	0.119	0.049
		Oct. 4, 1980	S80	95.5	0.095	0.050
Prince Edward Island, P.E.I.	47°10′N; 64°00W	Sept. 25, 1979	PEI79	96.2	0.119	0.048
Lurcher Shoals, N.S.	43°50′N; 66°30′W	Sept. 21, 1978	LS78	74.0	0.095	0.040
		Oct. 4, 1979	LS79	90.2	0.095	0.047
		Oct. 12, 1980	LS80	94.5	0.119	0.046
Jeffries Ledge, MA	42°40′N; 70°20′W	Oct. 2, 1978	JL78	79.8	0.095	0.050
		Oct. 1, 1979	JL79	102.8	0.119	0.053
		Oct. 5, 1980	JL80	98.6	0.117	0.050

[a]A locus is considered polymorphic if the frequency of the most common allele is ≤0.95.

areas; all fish were ripe. Samples of liver and muscle tissue were removed from individuals directly after capture and frozen in liquid nitrogen. Tissues were later transferred to an ultracold freezer (−80°C) and stored for up to 6 mo prior to electrophoresis. Samples of Pacific herring (*Clupea harengus pallasi*) in non-spawning condition were obtained from Puget Sound.

Tissues were homogenized in an equal volume of cold 10 mmol Tris-HCl, pH 7.0 (containing 5 mmol dithiothereitol and 0.5% polyvinylpyrrolidone-360), and centrifuged at 2000 × *g* for 5 min. Horizontal starch gel electrophoresis of supernatants followed the procedure outlined by May et al. (1979). For most proteins, histochemical staining followed the methods of Shaw and Prasad (1970) and Harris and Hopkinson (1976). Aspartate aminotransferase was visualized by the method of Johnson et al. (1972). Of 38 proteins initially screened, 29 enzymes (Table 2) were examined for biochemical variants.

Designation of isozymes and genotypes follows the uniform nomenclature suggested by Allendorf and Utter (1979). For statistical interpretation of observed genetic variation, we employed contingency analysis of the log-likelihood procedure (Smouse and Ward 1978) adjusting the significance of probability tests for individual loci (Grant et al. 1980). Estimates of genetic similarites among sampled populations were calculated with the formulae of Nei (1972).

Results

We electrophoretically resolved 42 gene loci encoding 29 proteins. All of these loci were initially examined in a sample of 100 individuals from a single population (Jefferies Ledge) to characterize polymorphisms for subsequent population screening. Of the 20 proteins that exhibited monomorphic loci in this sample, 11 were electrophoretically compared with samples of Pacific herring. In this comparison, 9 of these 11 proteins exhibited divergent alleles at a minimum of one locus per protein. Thus, we are confident that our techniques were capable of detecting a significant proportion of electrophoretic variation that might have been present in our initial screening.

Electrophoretic variants were observed at 13 loci encoding nine enzymes (Fig. 1). However, variation was rare at many of these loci. Allelic frequencies for the most informative loci, i.e. those where the frequency of most common allele ≤ 0.95, are presented in tables 3 and 4. Allelic frequencies for all other variable loci are available from the authors. A brief characterization of each polymorphic system follows.

ACON — *ACON* is encoded by two anodal loci of which the more anodal, *ACON-2*, was polymorphic and possessed five alleles. Two-banded heterozygotes implied monomeric structure of the active isozyme. Care must be taken in staining because the linking enzyme in the stain is very sensitive to heat denaturation; *ACON* itself is quite labile. Variation for *ACON* has not been previously reported in Atlantic herring.

AAT — A single anodal locus segregating for three alleles was observed. A ratio of 1:2:1 staining intensities observed in the three-banded heterozygote phenotypes implies that the locus is dimeric. Crosses among *AAT* homozygotes produced only homozygous offspring (Kornfied et al. 1981).

TABLE 2. Proteins resolved and electrophoretic methods for *Clupea harengus*.

Protein	No. of loci	Tissue	Buffer[a]
Aconitase (*ACON*: EC 4.2.1.3)	2	Liver	3
Aspartate aminotransferase (*AAT*; EC 2.6.1.1)	1	Muscle	1
Alcohol dehydrogenase (*ADH*; EC 1.1.1.1)	2	Liver	3
Alphaglycerophosphate dehydrogenase (*AGP*; EC 1.1.1.8)	3	Muscle	2
Creatine kinase (*CPK*; EC 2.7.3.2)	2	Muscle	3
Esterase (*EST*; EC 3.1.1.1)	1	Liver	1
Fumerase (*FUM*; EC 4.2.1.2)	1	Muscle	3
Fructose diphosphatase (*FDP*; EC 3.1.3.11)	1	Muscle	2
Glucoaminidase (*GA*)	2	Liver/muscle	1
Glutamate dehydrogenase (*GDH*; EC 1.4.1.2)	1	Muscle	3
Glyceraldehyde-phosphate dehydrogenase (*GAP*; EC 1.2.1.12)	2	Liver/muscle	2
Glutamic-pyruvate transaminase (*GPT*; EC 2.6.1.2)	2	Muscle	3
Hexokinase (*HK*; EC 2.7.1.1)	1	Liver/muscle	2
Isocitrate dehydrogenase (*IDH*; EC 1.1.1.41)	2	Liver/muscle	2
Lactate dehydrogenase (*LDH*; EC 1.1.1.27)	3	Muscle	1
Leucine dehydrogenase (*LeuDH*; EC 1.4.1.9)	2	Muscle	2
Monoamine oxidase (*MO*; EC 1.4.3.4)	1	Muscle	4
Malic enzyme (*ME*; EC 1.1.1.40)	2	Muscle	2
Methylumbelliferyl phosphatase (*MUP*: EC 3.1.3.2)	2	Liver/muscle	4
Nonspecific target stain (*NSG*)	1	Muscle	1
Peptidase (*PEP*; EC 3.4.11)	1	Muscle	3
Phosphoglucose isomerase (*PGI*; EC 5.3.1.8)	2	Muscle	1
Phosphoglucomutase (*PGM*; EC 2.7.5.1)	2	Muscle	1
Phosphomannose isomerase (*PMI*; EC 5.3.1.8)	1	Liver	1
6-Phosphogluconate dehydrogenase (*6PG*; EC 1.1.1.44)	1	Muscle	2
Superoxide dismutase (*SOD*; EC 1.15.1.1)	1	Liver	1
Sorbitol dehydrogenase (*SDH*; EC 1.1.1.14)	1	Muscle	1
Triosephosphate isomerase (*TPI*; EC 5.3.1.1)	1	Liver	2
Xanthine dehydrogenase (*XDH*; EC 1.2.1.37)	1	Liver	2

[a]Electrophoretic buffers: (1) LiOH, pH 8.1 (Ridgway et al. 1970); (2) *N*-(3-aminopropyl)-morpholine, pH 6.1 (Clayton and Tretiak 1972); (3) Tris–borate–EDTA, pH 8.7 (Market and Faulhaber 1965); (4) Tris–citrate–EDTA, pH 7.0 (Ayala et al. 1974). Electrode buffer 4 was diluted 1:15 for gel buffer.

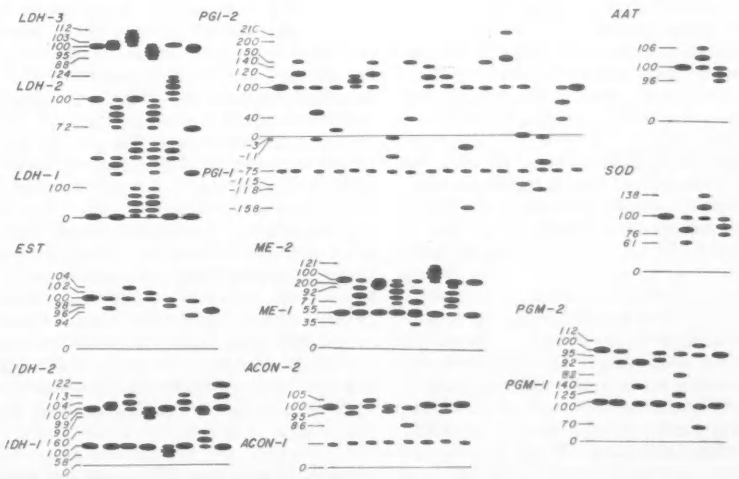

FIG. 1. Observed electrophoretic phenotypes for all variable loci in Atlantic herring. Anode is above the origin in all zymograms.

TABLE 3. Allele frequencies of four polymorphic loci in Atlantic herring.

Sample		*ACON-2*						*EST*		
		N	*100*	*95*	*86*	*105*	*60*	*N*	*100*	*96*	*104*
Spring spawners											
Carleton	1979	44	0.932	0.068	0.0	0.0	0.0	43	0.802	0.198	0.0
	1980	62	0.863	0.121	0.008	0.0	0.008	63	0.794	0.198	0.008
Ile Verte	1979	67	0.896	0.060	0.022	0.022	0.0	60	0.725	0.258	0.008
	1980	98	0.832	0.123	0.026	0.020	0.0	98	0.801	0.179	0.015
Fall spawners											
Miscou	1979	47	0.787	0.213	0.0	0.0	0.0	47	0.670	0.309	0.021
Stonehaven	1979	41	0.793	0.183	0.024	0.0	0.0	38	0.776	0.224	0.0
	1980	96	0.839	0.141	0.020	0.0	0.0	96	0.781	0.214	0.005
Prince Edward Island	1979	98	0.867	0.107	0.020	0.005	0.0	97	0.773	0.201	0.016
Lurcher Shoals	1978	74	0.878	0.115	0.0	0.067	0.0	74	0.716	0.284	0.0
	1979	90	0.811	0.183	0.006	0.0	0.0	90	0.689	0.294	0.011
	1980	95	0.879	0.111	0.005	0.005	0.0	94	0.761	0.239	0.0
Jefferies Ledge	1978	81	0.840	0.154	0.006	0.0	0.0	79	0.633	0.348	0.013
	1979	89	0.758	0.219	0.223	0.0	0.0	104	0.668	0.313	0.014
	1980	96	0.813	0.172	0.100	0.005	0.0	100	0.690	0.300	0.005

Variation at this locus has been previously reported in Atlantic herring from eastern North America and western Europe (Odense et al. 1966, 1973; Andersson et al. 1981).

EST — Esterase is coded for by several loci, only the most anodal of which was interpretable. This fast locus was polymorphic with six alleles observed. The presence of two-banded heterozygotes implied a functional monomer. Crosses among *EST* homozygotes produced only homozygous progeny (Kornfield et al. 1981). Variation of esterase in Atlantic herring has previously been reported (Naevdal 1969b; Simonarson and Watts 1969; Ridgway et al. 1970; Odense et al. 1973; Andersson et al. 1981).

IDH — Two anodal polymorphic loci were observed. We have demonstrated Mendelian inheritance of allelic variation for *IDH-2* (Kornfield et al. 1981). Variation at *IDH* in Atlantic herring has been reported by Wolf et al. (1970) and Andersson et al. (1981).

LDH — Three loci were observed; however, only two of these (*LDH-1* and *LDH-2*) were scored in all populations. Mendelian inheritance of alleles has been demonstrated for both of these loci (Kornfield et al. 1981). At *LDH-1*, the common allele is situated on the origin of the gel and is assigned mobility *0*. Variation at *LDH* in the Atlantic subspecies has been previously reported (Odense et al. 1966, 1973; Klose et al. 1968; Andersson et al. 1981).

ME — Allelic variants were observed at two anodal loci. Mendelian inheritance has been established for *ME-2* (Kornfield et al. 1981). Tetrameric structure is consistent with the observed heterozygote phenotype. A rare variant of *ME-2* (*55*) was equivalent in mobility to the common *100* allele at *ME-1* (Fig. 1). Variation at *ME-2* was previously reported in Atlantic herring (Andersson et al. 1981).

PGI — One polymorphic anodal locus (*PGI-2*) and one monomorphic cathodal locus (*PGI-1*) were studied. Allelic variation at *PGI-2* is inherited in a Mendelian manner (Kornfield et al. 1981). The single *PGI-1* allele was equivalent in mobility to the *−75* allele of *PGI-2*. No heterodimers occur between loci. *PGI-2* was highly polymorphic exhibiting a total of 14 anodal and cathodal alleles throughout the range studied. Many of these alleles were rare variants restricted to single localities. Two null alleles were encountered in herring sampled from Miscou. Compared to the 1:2:1 staining intensities of normal three-banded heterozygotes, null heterozygotes were recognized as phenotypes which exhibited two bands of equal staining intensity representing the normal/normal homodimer and the normal/null heterodimer. Mobilities of null alleles were calculated as twice the distance between the normal homodimer and the normal/null heterodimer; null homodimers are represented by dotted lines in Fig. 1. Variation at *PGI-2* in Atlantic herring has been previously described (Lewis and Ridgway 1972; Odense et al. 1973; Schmidtke et al. 1975; Andersson et al. 1981).

PGM — Two polymorphic anodal loci were studied. Both loci exhibited Mendelian inheritance of allelic variants (Kornfield et al. 1981). Variation in *PGM* has been described

			LDH-2				*PGM-2*					
98	*102*	*94*	*N*	*100*	*72*	*124*	*N*	*100*	*92*	*112*	*82*	*95*
0.0	0.0	0.0	44	0.943	0.057	0.0	44	0.591	0.386	0.011	0.012	0.0
0.0	0.0	0.0	84	0.964	0.036	0.0	63	0.460	0.468	0.048	0.024	0.0
0.0	0.008	0.0	67	0.955	0.045	0.0	67	0.641	0.321	0.022	0.016	0.0
0.005	0.0	0.0	98	0.980	0.020	0.0	98	0.602	0.398	0.0	0.0	0.0
0.0	0.0	0.0	47	0.958	0.042	0.0	47	0.606	0.372	0.021	0.0	0.0
0.0	0.0	0.0	41	0.939	0.049	0.012	41	0.561	0.427	0.0	0.012	0.0
0.0	0.0	0.0	96	0.969	0.031	0.0	91	0.599	0.374	0.022	0.0	0.006
0.005	0.005	0.0	100	0.950	0.035	0.015	87	0.581	0.368	0.040	0.006	0.005
0.0	0.0	0.0	74	0.980	0.020	0.0	74	0.574	0.399	0.027	0.0	0.0
0.0	0.0	0.006	90	0.972	0.028	0.0	90	0.500	0.494	0.006	0.0	0.0
0.0	0.0	0.0	95	0.911	0.089	0.0	90	0.556	0.361	0.056	0.022	0.006
0.0	0.0	0.006	81	0.969	0.031	0.0	81	0.691	0.284	0.012	0.006	0.006
0.005	0.0	0.0	104	0.947	0.053	0.0	104	0.548	0.414	0.038	0.0	0.0
0.0	0.0	0.005	100	0.945	0.055	0.0	94	0.500	0.431	0.021	0.048	0.0

in western European samples of Atlantic herring (Lush 1969; Andersson et al. 1981).

SOD — A single anodal locus polymorphic for four alleles was studied. Crosses among *SOD* homozygotes produced only homozygous progeny (Kornfield et al. 1981). Variation for *SOD* was previously reported by Andersson et al. (1981).

Among the 12 samples, the average proportion of loci heterozygous per individual was 0.048 and average proportions of loci polymorphic per sample were 0.109 and 0.213 for the 95 and 99% polymorphism criteria, respectively (Table 1). With only a single exception, all samples were in Hardy–Weinberg–Castle equilibrium for all polymorphic loci. Observed genotypes for *EST* in the 1980 herring sample from Jefferies Ledge deviated significantly from equilibrium expectations (pooling alleles *102* and *104*, $\chi^2 = 11.53$, $df = 3$, $P < 0.01$).

Allele frequencies for loci polymorphic under the 95% criterion (*ACON-2*, *EST*, *LDH-2*, *PGI-2*, and *PGM-2*) were examined to estimate the magnitude of differentiation among herring populations by log-likelihood procedure (Smouse and Ward 1978). Tests associated with individual loci were summed and examined for overall significance. First, intralocality temporal variation was tested for all populations which were sampled in more than 1 yr (Table 5). Second, seasonal variation among populations was tested in a hierarchical manner. We examined variation at each locus (1) within years for spring and fall samples separately, (2) between spring and fall for specific years, and (3) between spring and fall among years (Table 6).

Significant intralocality temporal variation was found in 3 of the 5 multi-year population samples. Frequency variation was particularly pronounced among the Lurcher Shoals herring where temporal heterogeneity for 1978–80 was highly significant. Two loci, *EST* and *PGI-2*, exhibited no significant annual variation for any locality.

Among geographic samples within both spring and fall spawning populations, significant heterogeneity was observed at several individual loci, but, over the 3-yr analysis, no locus exhibited consistent differences among localities. Further, overall heterogeneity within spawning seasons was significant in some years but nonsignificant in others.

The comparison of spring and fall sampling populations revealed highly significant overall heterogeneity. In addition, the two loci which showed no temporal variation within localities (*EST* and *PGI-2*) exhibited significant heterogeneity between spring and fall spawners. It is not appropriate to interpret the significant difference between spring and fall samples without correcting for the variation which occurred within seasons. To characterize this difference, we generated an approximate F test as the ratio of (1) likelihood/degrees of freedom for the sum of all loci for all samples divided by (2) the pooled likelihood estimate/pooled degrees of freedom for the sum of all loci among spring spawning and among fall spawning samples. A highly significant overall difference between spring and fall spawning areas was revealed by this test ($F = 5.54$; $df = 10, 30$; $P < 0.001$).

Because of the spatial distribution of our samples, it was possible that latitudinal heterogeneity contributed to the significant variation observed between populations spawning in different seasons. This concern was particularly relevant as latitudinal gradients in allele frequency have been

TABLE 4. Allelic frequencies of *phosphoglucose isomerase-2* in Atlantic herring.

Sample		N	*100*	*−75*	*−3*	*150*	*−115*	*40*	*120*	*140*	*210*	*−11*	*200*	*140* (null)	*158*	*−118*
								Spring spawners								
Carleton	1979	44	0.727	0.091	0.057	0.091	0.023	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1980	77	0.656	0.091	0.078	0.149	0.020	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.007	0.0
Ile Verte	1979	67	0.687	0.105	0.112	0.082	0.008	0.008	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1980	98	0.735	0.097	0.082	0.082	0.0	0.005	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
								Fall spawners								
Miscou	1979	45	0.544	0.100	0.133	0.078	0.004	0.011	0.022	0.0	0.0	0.0	0.044	0.022	0.0	0.0
Stonehaven	1979	41	0.707	0.085	0.085	0.098	0.024	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1980	95	0.626	0.153	0.095	0.095	0.016	0.011	0.0	0.0	0.005	0.0	0.0	0.0	0.0	0.0
Prince Edward Island	1979	95	0.590	0.105	0.132	0.137	0.026	0.005	0.0	0.005	0.0	0.0	0.0	0.0	0.0	0.0
Lurcher Shoals	1978	74	0.669	0.149	0.108	0.061	0.014	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1979	89	0.663	0.118	0.101	0.112	0.0	0.0	0.006	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1980	95	0.674	0.126	0.105	0.068	0.021	0.0	0.0	0.0	0.005	0.0	0.0	0.0	0.0	0.0
Jeffries Ledge	1978	81	0.670	0.136	0.074	0.093	0.006	0.006	0.006	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1979	104	0.630	0.125	0.082	0.111	0.029	0.005	0.005	0.005	0.005	0.0	0.0	0.0	0.0	0.005
	1980	99	0.657	0.121	0.111	0.106	0.0	0.0	0.0	0.0	0.0	0.005	0.0	0.0	0.0	0.0

TABLE 5. Likelihood analysis of intralocality temporal variation. (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.)

	df	*ACON-2*	df	*EST*	df	*LDH-2*	df	*PGI-2*	df	*PGM-2*	df	SUM
					Fall spawners							
Stonehaven (1979–80)	2	0.83	1	0.01	1	1.22	4	2.92	2	1.19	10	6.17
Lurcher Shoals (1978–80)	4	17.44**	2	2.41	2	10.77*	8	6.48	4	20.46**	20	57.56***
Jefferies Ledge (1978–80)	4	4.48	2	1.29	2	1.47	8	11.64	4	15.95*	20	34.82*
					Spring spawners							
Ile Verte (1979–80)	2	3.82	1	2.40	1	1.42	4	1.88	2	10.51*	10	20.04*
Carleton (1979–80)	2	3.93	1	0.02	1	0.60	4	2.48	2	3.38	10	10.42

demonstrated for several teleosts (e.g. Koehn 1969; Place and Powers 1979). Regressions of arcsine square root transformed gene frequencies versus latitude were performed for the common allele of the five polymorphic loci identified above. Of the five, *EST* exhibited a significant linear cline with latitude (Fig. 2; $F = 10.98$; df = 1, 12; $P < 0.01$). Regardless, highly significant overall heterogeneity between spring and fall populations persisted even when *EST* was eliminated from the likelihood analysis.

Genetic similarities among samples calculated on the basis of the 13 variable loci all exceeded $I_N = 0.994$. To provide better characterization of intersample identity, we recalculated similarities based only on the highly polymorphic loci (*ACON-2*, *EST*, *PGM-2*, and *PGI-2*). Similarities were again very high ranging from 0.969 to 0.998 (Table 7).

Discussion

Attempts to define populations of Atlantic herring have had a long and controversial history. Although earlier workers considered spring and fall spawning herring to represent a single population (Jean 1956; Tibbo and Graham 1963), more recent studies have strongly supported the idea of separate stocks (Messieh and Tibbo 1971; Messieh 1975; Côté et al. 1980). In addition to spawning time, these two groups differ in age–frequency distribution, growth rate, otolith morphology, and mean number of pectoral and dorsal fin rays. Biologists have been less certain about the genetic uniqueness of individual spawning aggregations, but a few authors have suggested that genetic isolation exists between stocks (Ridgway et al. 1970; Lewis and Ridgway 1972; Zenkin 1978). However, because non-spawning fish were examined in many of these studies and characters which could potentially be environmentally influenced were employed, it is difficult to make a definitive test for genetic isolation. Our examination of inherited biochemical variation in spawning herring aggregations circumvented these weaknesses.

It is clear from the likelihood analysis of genetic variation (Table 6) that spring spawning herring are genetically differentiated from fall spawners; our electrophoretic results are congruent with the findings from morphological analyses and thus attest to the sensitivity of the genetic approach. It is of interest that the spring–fall difference was detectable in the face of significant annual variation. Fall and spring spawning herring possess isolated gene pools which can respond independently to ecological variation or fishing pressure. However, the magnitude of differentiation between these groups is small and implies that they are probably of relatively recent common origin. More importantly, statistically significant differences between seasonal spawners do not provide suitable biochemical markers for accurate discrimination of individual fish. Statistical separation also does not preclude limited gene flow between populations spawning in different seasons. For example, while morphological discriminate functions convincingly separated fall and spring spawning herring examined by Côté et al. (1980), ~5% of spawning individuals was misclassified. Such an error could indicate the magnitude of gene flow between seasonal isolates, though other factors probably contribute to incorrect identification. The absence of consistent heterozygote deficiencies at polymorphic loci within spring spawning samples also suggests exteremely little interseasonal gene exchange. Nevertheless, the extent of gene flow must be very small, or genetic differences could not persist (Aspinwall 1974; Allendorf and Phelps 1981).

The existence of additional genetically isolated or semi-isolated populations within spring or fall spawning groups is less clear. Because populations are so similar (Tables 3 and 4), simple statistical comparisons of gene frequencies among samples within seasons are nonsignificant. The yearly variation in gene frequencies of *ACON-2*, *LDH-2*, and *PGM-2* at specific sampling sites (Table 5) masks differences that might exist among spatially separate spawning localities. Our findings emphasize the importance of collecting multi-year data in general isozyme studies. Had we only sampled in 1978 or in 1979, we would have interpreted significant heterogeneity among fall spawning locations as indicative of several genetically isolated populations. However, because of homogeneity among samples collected from the same spawning localities in 1980 (Table 6), it is clear that spatial heterogeneity is temporally unstable. Thus, given the irregular nature of this variation, it was not possible for us to demonstrate isolation among specific geographic samples within spawning seasons.

It is difficult for us to localize the source of temporal variation. Population sizes of herring are enormous (Iles and Sinclair 1982) and massive spawnings would suggest the absence of population subdivision at specific spawning localities. Thus, for selectively neutral markers, we would expect that gene frequencies would be stable over relatively long periods of time (Roughgarden 1979). Though a stochastic component is, of course, present, it should currently contribute little to either temporal or spatial differentiation over

TABLE 6. Likelihood analysis of of allelic frequencies in Atlantic herring. (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.)

Source of variation	df	*ACON-2*	df	*EST*	df	*LDH-2*	df	*PGI-2*	df	*PGM-2*	df	SUM
Between spring and fall spawners (total)	2	16.85**	1	9.33*	1	1.23	4	85.18**	2	2.88	10	115.47***
Among fall spawners (1978)	2	9.98*	1	2.42	1	0.35	4	2.61	2	4.69	10	20.05*
Between spring and fall (1979)	2	20.29**	1	1.75	1	0.05	4	5.97	2	3.72	10	31.77***
Among spring spawners (1979)	2	6.20	1	1.65	1	0.16	4	3.02	2	1.24	10	12.27
Among fall spawners (1979)	8	16.38	4	8.44	4	2.26	16	33.03*	8	15.08	40	75.18***
Between spring and fall (1980)	2	3.61	1	3.52	1	5.18	4	6.47	2	9.96*	10	28.74**
Among spring spawners (1980)	2	2.27	1	0.03	1	0.79	4	7.07	2	14.97**	10	25.13**
Among fall spawners (1980)	4	3.71	2	4.65	2	6.00	8	7.64	4	8.68	20	30.68

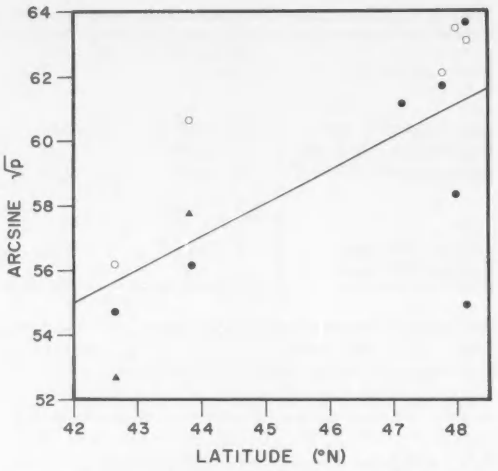

FIG. 2. Linear regression of arcsine squareroot EST^{100} frequency on north latitude. Arcsine $\sqrt{p} = 11.18 + 1.04$ latitude. Squares, solid circles, and open circles represent samples from 1978, 1979, and 1980, respectively.

a period of decades. Herring have overlapping generations and can be reproductively active for several years, so it is doubtful that intergenerational or year-class differences contribute significantly to population differentiation; the absence of significant departures from Hardy–Weinberg–Castle expectations is consistent with this idea. Changing selection pressures could produce significant temporal variation, but the presence of selection is undocumented and extremely difficult to assess. The latitudinal cline in esterase gene frequency (Fig. 2), for example, can be explained both by selective or nonselective processes, e.g. historical patterns of gene flow. One probable cause for a portion of the temporal variation is simple sampling error. The sizes of most samples in our study are large enough to demonstrate reliably major changes in gene frequency, but confidence limits are sufficiently broad that they encompass the range of observed frequency variation. Thus, some of the observed temporal variation may be more apparent than real.

On the basis of gene frequency differences at *EST* and *PGI-2* in non-spawning herring, Ridgway et al. (1970) and Lewis and Ridgway (1972) suggested the existence of separate herring subpopulations in the Gulf of Maine. It is of interest that *PGI-2* is one of several polymorphic loci in herring (including *AAT*, *IDH-2*, *LDH-1*, and *LDH-2*) which exhibit no significant frequency differences between western Europe and eastern North America (Wolf et al. 1970; Odense et al. 1973). Given oceanic homogeneity for *PGI-2* and clinal variation in *EST* (in addition to the errors associated with comparing non-spawning herring from a single year), a conclusion of genetic isolation seems unjustified. Previously, Anthony and Boyar (1968) noted significant differences in pectoral ray and vertebral number among samples of Atlantic herring from the northwest Atlantic. They concluded that two general complexes of herring exist within

TABLE 7. Genetic similarities (I_N) between samples of Atlantic herring for *ACON-2*, *EST*, *PGI-2*, and *PGM-2*. Sample abbreviations in Table 1.

C80	0.993															
IV79	0.996	0.984														
IV80	0.997	0.992	0.994													
JL78	0.983	0.969	0.993	0.983												
JL79	0.985	0.987	0.986	0.989	0.989											
JL80	0.989	0.993	0.989	0.991	0.985	0.997										
LS78	0.994	0.989	0.996	0.993	0.990	0.993	0.995									
LS79	0.987	0.993	0.984	0.989	0.980	0.997	0.998	0.993								
LS80	0.997	0.993	0.996	0.996	0.987	0.991	0.994	0.997	0.991							
M79	0.982	0.980	0.987	0.984	0.990	0.995	0.991	0.991	0.989	0.989						
PEI79	0.995	0.993	0.994	0.994	0.983	0.988	0.990	0.994	0.988	0.997	0.990					
S79	0.991	0.991	0.987	0.995	0.979	0.991	0.992	0.989	0.992	0.991	0.986	0.990				
S80	0.995	0.992	0.995	0.997	0.987	0.992	0.993	0.996	0.990	0.998	0.991	0.998	0.992			
	C 79	C80	IV79	IV80	JL78	JL79	JL80	LS78	LS79	LS80	M79	PEI79	S79			
	Spring spawners							Fall spawners								

the Gulf of Maine. Meristic differences observed by them between the 2 yr sampled were interpreted as indicating a change in the distribution of herring. However, variation in meristic characters may be induced by different thermal regimes during development (Day 1957; Ali and Lindsey 1974; Dentry and Lindsey 1978). We suggest that the absence of temporal consistency in meristic data does not support a multipopulation model.

Our results are not consistent with the existence of more than a single population of fall spawning herring in the Gulf of Maine. This finding contrasts with that in the more polymorphic Pacific subspecies where significant geographic heterogeneity has been identified (W. S. Grant, Northwest & Alaska Fisheries Center, NMFS, Seattle, WA 98112, USA, personal communication). While it is possible that spawning aggregations of Atlantic herring are reproductively isolated within a particular season, the genetic differences among populations must be extremely small. To identify reproductive isolation, if in fact it exists, we suggest that future research might emphasize tagging programs on spawning fish or comparative analyses of rapidly evolving genetic material, such as mitochrondrial DNA.

Acknowledgments

The Stinson Canning Co., Peacock Canning Co., and Atlantic Herring Fisheries Marketing Cooperative generously provided ship time and herring specimens for analysis. We thank Dave Smith, Bruce Cyr, Chuck Jagoe, and Matt Schardt for assistance in field collections. Howard Powles (Environment Canada) and V. Chaison kindly supplied us with much information and logistical support in addition to valuable discussion. In the course of this work, we benefitted from conversations with Guy Marshesseault, Vaughn Anthony, George Ridgway, Ken Honey, and Dick Hennemuth. Stew Grant kindly provided samples of Pacific herring. Jon Stanley critically commented on a draft of this manuscript. Daphne Fairbairn in review made valuable comments on data analysis. Our work was supported by NOAA Sea Grant No. NA 80AAD00034, NSF grant DEB 78-24074, and a grant from the Maine Sardine Council.

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Macroinvertebrate Colonization of Multiplate Samplers in the Ohio River: the Effect of Dams

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BECKETT, D. C., AND M. C. MILLER. 1982. Macroinvertebrate colonization of multiplate samplers in the Ohio River: the effect of dams. Can. J. Fish. Aquat. Sci. 39: 1622 – 1627.

We investigated the importance of contrasting current velocities on macroinvertebrate colonization in a large river, manipulating multiplate samplers placed upstream (slow current) and downstream (fast current) from a surface-release dam. Some samplers were colonized in solely fast-water or slow-water conditions while other colonized samplers were "switched" (with the original communities intact) to the opposite condition and subsequently collected either 1 d or 1 wk later. Experimental results confirmed observations from an earlier length-of-the-river study and showed that widely different communities become established on the samplers in the two contrasting flow conditions. An amphipod, *Gammarus* sp., colonized slow-water samplers in relatively large numbers while hydropsychid caddisflies dominated fast-water samplers. *Gammarus* sp. was found in only very small numbers in the fast-water samplers while the hydropsychids were almost completely absent from the slow-water samplers. Similar striking differences in current velocity preferences were exhibited by the congeneric chironomid species *Polypedilum convictum* (fast-water) and *P. illinoense* (slow-water). The transfer portion of the experiment demonstrated that a sudden reduction in current will cause large increases in invertebrate drift, regardless of whether the amount of living space changes.

Key words: freshwater macroinvertebrates, colonization, current, invertebrate drift, impoundment effects, Ohio River

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L'importance de vitesses de courant qui contrastent sur la colonisation des macro-invertébrés dans une grande rivière a été étudiée par manipulation d'échantillonneurs à plaques multiples placés en amont (faible courant) et en aval (fort courant) d'un barrage à écoulement de surface. Certains échantillonneurs ont été colonisés soit dans le fort courant, soit dans le faible courant seulement, alors que d'autres, déjà colonisés et transférés soudainement (les communautés originelles étant intactes) dans le courant opposé ont été ensuite été retirés une journée ou une semaine plus tard. Les résultats expérimentaux confirment les observations d'une étude antérieure menée tout long de la rivière. Ils démontrent que des communautés différentes s'établissent sur les échantillonneurs dans des conditions opposées de débit. Un amphipode, *Gammarus* sp., a colonisé les échantillonneurs en eau lente en nombres relativement grands, alors que les phryganes hydropsychides dominent dans les échantillonneurs placés en eau rapide. On trouve *Gammarus* en très petits nombres dans les échantillonneurs placés en eau rapide, alors que les hydropsychides sont presque complètement absents des échantillonneurs d'eau lente. Les espèces congénériques de chironomides, *Polypedilum convictum* (eau rapide) et *P. illinoense* (eau lente) montrent les mêmes différences dans leur préférence de vitesse de courant. La partie transfert de l'expérience démontre qu'une diminution soudaine de courant causera de fortes augmentations de dérive d'invertébrés, qu'il y ait ou non variation de l'espace vital disponible.

Received May 11, 1982
Accepted August 27, 1982

Reçu le 11 mai 1982
Accepté le 27 août 1982

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Printed in Canada (J6928)
Imprimé au Canada (J6928)

AN unfortunate consequence of the impoundment of rivers and streams is the elimination or reduction in numbers of some macroinvertebrate taxa in lotic habitats downstream of the impoundment structure (Hilsenhoff 1971; Spence and Hynes 1971; Fisher and LaVoy 1972; Lehmkuhl 1972; Ward 1976; Gore 1977; Armitage 1978). One of the principal causative factors of these effects is the hypolimnetic release from the impounded reservoir altering downstream temperature regimes (Pearson et al. 1968; Hilsenhoff 1971; Spence and Hynes 1971; Lehmkuhl 1972; Ward 1976; Gore 1977). A number of lotic systems which have been impounded do not show such temperature effects, however, because: (1) enough flow and turbulence remain through the system to preclude stratification; and (2) water release from the reservoir may be over the top of the dam structure or immediately below the surface. Impoundments of this type are used for a number of purposes including the maintenance of a minimum year-around navigational channel (e.g. the Ohio R., the upper Mississippi R., the Arkansas R., and other impounded navigable rivers).

Regardless of the type of water release (deep release or surface), the damming of rivers may have profound effects on the distribution of the rivers' biotas. An investigation in 1978 conducted by D.C.B. over the length of the Ohio R. (surface release dams) using artificial substrate (multiplate) samplers showed macroinvertebrate communities immediately upstream of the dams to be markedly dissimilar from communities at sites in which a greater current was maintained (Table 1). This suggested a cause-and-effect relationship in which the presence and abundances of various taxa were largely a function of current.

In this study we have manipulated macroinvertebrate communities on artificial substrates in the Ohio R. (1) to test the hypothesis that faunal distribution in large rivers can be a function of current; (2) to determine the organisms' relative rates of immigration and emigration from substrates which had been colonized in one current regime but were then transferred to another; (3) to test in a large river the current-dependent responses of hydropsychid caddisflies which had been previously observed in laboratory artifical stream studies (Philipson 1969) or small streams (Edington 1968); (4) to investigate the contention that a sudden reduction in water flow will result in an increase in invertebrate drift (Pearson and Franklin 1968; Minshall and Winger 1968; Gore 1977).

Methods

STUDY AREA AND FIELD METHODS

The Ohio R. is formed by the confluence of the Allegheny and Monongahela rivers at Pittsburgh, PA, and flows 1578 river km in a southwesterly direction joining the Mississippi R. at Cairo, IL. River discharge at Meldahl Locks and Dam, our experimentation site in autumn of 1979, ranged over the year (1979) from 969 m^3/s (July) to 15 451 m^3/s (March). The river has been impounded by a lock-and-dam system to maintain a year-around minimum navigational channel. At present 20 sets of lock-and-dam structures modify flow in the Ohio so that the river forms, at low or moderate flows, a repeatable flow pattern for each of the river's 20 "pools." Areas downstream from dams are shallow with a swiftly moving current; current gradually slows as the water nears the dams and river depth increases (see Fig. 1). Generally the river also widens as it approaches the dams, increasing the cross-sectional area of the river and further attenuating current such that current velocity is very slow immediately upstream of the dams.

This experiment was conducted at the Meldahl Locks and Dam located at river km 701.8, impounding a pool of 153.2 km. Water is released from the dam slightly below the river's surface. In autumn of 1979 we suspended 12 masonite multiplate samplers at a depth of 0.7 m from a float placed immediately upstream of Meldahl Dam (slow current), while another float, also with a set of 12 multiplate samplers suspended at 0.7 m, was placed ca. 0.8 km downstream of (below) the dam (fast current). The multiplate samplers were similar to those described by Fullner (1971). Artificial substrates were an ideal means of sampling because each sampler presented an identical area and substrate for colonization (Beak et al. 1973; United States Environmental Protection Agency 1973). Sampler positions were numbered on the top of the triangular floats so that each sampler could be identified and its manipulative treatment recorded.

Some of the above-dam and below-dam samplers were collected for analysis after a 4-wk colonization period. On the same day that the 4-wk collections were made other samplers were moved (from both sites) in tight-fitting containers and carefully resuspended (with the original communities intact) from floats in current conditions opposite to that in which they had originally been colonized. Some of these "switched" samplers were then collected after 1 d at their respective new sites while other "switched" samplers were collected after 1 wk exposure to the new conditions. Finally, four above-dam and four below-dam samplers which had experienced a 5-wk colonization over the study period and had not been "switched" were then collected (Table 2).

On the same date the 5-wk colonized samplers were collected, controls were run to determine if placement of the samplers into the containers, transport to the new site, and placement into the water had disrupted or changed the communities on the multiplates. The controls (two samplers from both slow-water and fast-water conditions which had a 5-wk colonization period) were removed from the water, placed in tight-fitting containers, and transported to the other condition site. These samplers were then removed from their containers, immersed in the river for ca. 3 s, and then pulled out of the water and placed in new containers. The controls could therefore be compared to the respective samplers which had been colonized over the same 5-wk period but had been retrieved without transport to, and immersion in, the other current conditions. A summary of experimental treatments and the terminology used to describe these treatments is presented in Table 2.

Samplers were taken to the laboratory where they were disassembled, surfaces "scraped" using a small brush, and macroinvertebrates collected using a U.S. Standard 60 sieve (openings = 250 μm).

STATISTICAL ANALYSIS

We used the Wilcoxon Rank Sum Test (Remington and Schork 1970) (also called Wilcoxon's two-sample test) to

TABLE 1. Summary of *Polypedilum* distribution in the length of the Ohio R. study (1978).

Station	River km	No. of *P. illinoense*	% of total *Polypedilum* spp. individuals consisting of *P. illinoense*	No. of *P. convictum*	% of total *Polypedilum* spp. individuals consisting of *P. convictum*
			Locks and dam sampling stations		
Belleville Dam	328.1	71	89	8	10
Greenup Dam	548.7	23	88	3	12
Meldahl Dam	701.8	3	100	0	0
Markland Dam	855.2	7	100	0	0
Cannelton Dam	1159.6	59	98	0	0
Uniontown Dam	1361.2	38	93	3	7
			Non-locks and dam sampling station		
Ohio R. at Joppa, IL	1532.2	11	10	98	90

determine the current velocity preferences of the more commonly collected taxa. The number of animals collected on each sampler for the 5-wk fast-water treatments ($F_5 + F_c$) were compared to the numbers present on each sampler from the 5-wk slow-water treatments ($S_5 + S_c$) for each of the common species.

Results

The Ohio R. study of autumn 1978 shows an interesting contrast in macroinvertebrate fauna at dam sites vs. non-dam sites (Table 1). Sampling at dam sites (all dams sampled on the upstream–slow current side) with multiplate samplers showed the larval chironomid *Polypedilum illinoense* to be the most common *Polypedilum* species at these sites. Its congener, *P. convictum*, a common chironomid in large river systems, was relatively scare at these sites. Sampling sites at dams were also characterized by the almost complete absence of larval *Hydropsyche orris*, a very common hydropsychid caddisfly in large river systems (Ross 1944; Schuster and Etnier 1978). Data from Joppa, Illinois, at an Ohio R. sampling site which possessed a fairly strong current, showed a marked contrast to the dam-site macroinvertebrates. At this site *P. convictum* was more abundant than *P. illinoense*, and *H. orris* was also very abundant.

Results from the Meldahl Dam experiment (Table 3) have confirmed the relationships discussed above. The most abundant non-chironomid macroinvertebrate colonizing the slow-water samplers upstream of the dam was the amphipod *Gammarus* sp. (an undescribed species near *G. fasciatus*) while hydropsychid caddisflies (mostly *H. orris*) were abundant in the downsteam collections. *Gammarus* sp. was found only in very small numbers in the fast-water samplers whereas the hydropsychids were almost completely absent from the slow-water samplers. The congeneric chironomid species *P. illinoense* and *P. convictum* presented a similar striking contrast with *P. illinoense* much more abundant in slow-water conditions and *P. convictum* numerically dominant in the fast-water samplers. A comparison for each of the more commonly collected species between their numbers per sampler for the 5-wk fast-water treatments vs. numbers collected for the 5-wk slow-water samplers showed that the winter stonefly *Taeniopteryx burksi*, *Gammarus* sp., and *P. illinoense* were significantly more abundant on the slow-water samplers (Table 3). In contrast, the larval chironomids *Cricotopus bicinctus*, *P. convictum*, and *Rheotanytarsus* sp., and the caddisfly *H. orris* were present in significantly greater numbers on the fast-water samplers.

A critical factor in the success of the manipulative portion of the experiment was the successful transplant of the samplers from one condition (slow or fast water) to the other, i.e. communities had to remain relatively intact on the samplers during the transfer process. Table 3 shows S_c results to be quite similar to S_5 both in terms of the total numbers of animals per sampler and the relative abundances of the various taxa which were collected. Likewise results from F_c and F_5 are very similar indicating that communities on transferred samplers were intact immediately after being switched to their new conditions.

A comparison of the $S–F_1$ to $S–F_7$ samplers shows that with increasing time of exposure to the fast water, *P. convictum* became more abundant whereas *P. illinoense* numbers descreased (Table 3). Likewise the number of *H. orris* per sampler increased slightly while the number of amphipods continued to decrease. This reflects a progressional trend as animals on the $S–F_7$ sampler showed increased similarity to the fast-water colonized communities. The reciprocal transfer $F–S_7$ shows that a complete shift in numerical dominance from *H. orris* larvae to *Gammarus* sp. has taken place as a consequence of the sampler placement into the slow current condition.

An interesting contrast exists between the $S–F$ and $F–S$ transfers in terms of changes in numbers of *Gammarus* sp. and *H. orris*. The $F–S$ transfers exhibited a much greater change for both the 1-d and 7-d transfers than the respective $S–F$ samplers (Table 3). This can be explained by examining the various species' relative rates of immigration and emigration to and from transferred samplers. *Gammarus* sp. colonized samplers transferred into slow water much faster than *H. orris* colonized samplers transferred into fast water ($F–S_1$: $\bar{x} = 4.5$ *Gammarus* per sampler and $F–S_7$: $\bar{x} = 16.0$ *Gammarus* per sampler while $S–F_1$: $\bar{x} = 0.5$ *H. orris* per sampler and $S–F_7$: $\bar{x} = 2.0$ *H. orris* per sampler). Both *Gammarus* sp. and *H. orris* left unfavorable habitats although *H. orris* emigrated somewhat more rapidly and completely. Mean number of *Gammarus* per sampler for the slow-water treatments ($S_4 + S_5 + S_c$) equaled 23.0 and was reduced to 9.5 in $S–F_1$ samplers (a 59% reduction) and finally to 3.0 in $S–F_7$ samplers (87% reduction). Mean number of *H. orris* for the fast-water treatments ($F_4 + F_5 + F_c$) equaled 11.5 and was reduced to 3.5

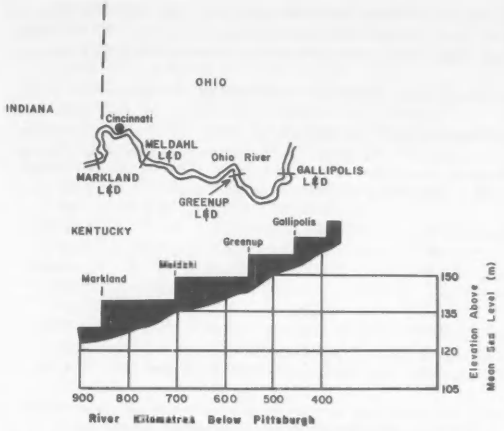

FIG. 1. Profile of Ohio R. pools near Meldahl Locks and Dam (L&D) showing repeated pattern of increased depth with the river's approach to the dams. Figure is based on a U.S. Army Corps of Engineers illustration.

in $F-S_1$ (a 70% reduction) and finally to 0.5 in $F-S_7$ (a 96% reduction).

Temperatures, dissolved oxygen, current velocity, and conductivity were measured at the sampler locations above and below the dam on several occasions. No temperature differences were detected, due to the near-surface release of water and river turbulence. Oxygen was always slightly higher below the dam, but was always near saturation both above and below the dam. Conductivity measurements below the dam were always within 3% of the above-dam readings. Current velocity varied depending on river discharge and was always much higher below the dam (range = 20–40 m/min) than above the dam (range = 0–10 m/min).

Discussion

Pearson and Franklin (1968), Minshall and Winger (1968), and Gore (1977) observed large increases in invertebrate drift in streams that underwent a sudden reduction in discharge. Although they concluded that the change in current could cause this response, in that conditions were no longer physiologically or behaviorally favorable to rheophilic organisms, their results were complicated by a large reduction in living space (especially in shallower areas) with decreased discharge. Water level reductions in Minshall and Winger's study amounted to 25–50%; in Gore's study the river was reduced from an average width of 30 m to 7 m; in Pearson and Franklin's study 25% of the previously submerged streambed was exposed. Minshall and Winger, therefore, had to conclude that "resultant crowding and jostling of the animals could cause an increase in activity and be responsible for their entry into the current." Our study shows a marked decrease in the abundance of the rheophilic species such as *C. bicinctus*, *P. convictum*, *Rheotanytarsus* sp., and *H. orris* in a comparison of the samplers transferred from fast to slow water

TABLE 2. Treatments of artificial substrate samplers in Meldahl Dam experiment. All samplers placed in river on Oct. 1, 1979.

Treatment code	Method of treatment	Date samplers collected
S_4	In slow water for 4 wk	Oct. 29, 1979
S_5	In slow water for 5 wk	Nov. 05, 1979
S_c	In slow water for 5 wk, then transferred and briefly immersed in fast water (S-control)	Nov. 05, 1979
F_4	In fast water for 4 wk	Oct. 29, 1979
F_5	In fast water for 5 wk	Nov. 05, 1979
F_c	In fast water for 5 wk, then transferred and briefly immersed in slow water (F-control)	Nov. 05, 1979
$S-F_1$	In slow water for 4 wk, then transferred to fast water for 1 d	Oct. 30, 1979
$S-F_7$	In slow water for 4 wk, then transferred to fast water for 7 d	Nov. 05, 1979
$F-S_1$	In fast water for 4 wk, then transferred to slow water for 1 d	Oct. 30, 1979
$F-S_7$	In fast water for 4 wk, then transferred to slow water for 7 d	Nov. 05, 1979

($F-S_1$) and the fast-water colonized samplers (F_4, F_5, and F_c) (Table 3). This reduction in the abundance of the rheophilic species took place in a fairly short time (1 d) and occurred in a system which was not complicated by reduction in the size of the habitat. We conclude, then, that such a response (mass emigration) can occur from the solitary causation of reduced current.

The absence of the hydropsychid caddisfly *H. orris* from slow current areas in this large river system is consistent with studies of various *Hydropsyche* spp. in a large river (Fremling 1960), in artificial streams (Edington 1969; Philipson 1969; R. L. Fuller, Colgate University, Hamilton, NY, personal communication), and in a small natural stream (Edington 1968). In these studies hydropsychids moved (drifted) out of reduced current areas or were present in markedly reduced numbers in such habitats. Edington (1968) placed a baffle in a small stream, creating a reduced current situation very similar to that of a dam. Counting the larvae before and after placement of the baffle, he noticed a reduction from 125 to 4 larvae in a 0.19 m^2 (2 ft^2) region behind the baffle in 43 h. Our experiment showed a similar reduction in hydropsychid numbers after a 24-h exposure to a reduced current velocity regime. Because hydropsychids are dependent upon current both for respiration (Philipson 1954) and for food gathering (Edington 1968; Wallace 1975a, b; Wiggins 1977), they will not colonize unfavorable areas and will move out of areas in which conditions become unfavorable. The damming of large rivers therefore creates some slow-water habitats which are no longer colonizable by *H. orris*.

Although such slow-water areas may no longer be suitable for *H. orris*, it is apparent that the amphipod *Gammarus* sp. readily and quickly exploits substrates in reduced current areas (Table 3). Other field observations (D. C. Beckett unpublished data) have shown that the Ohio R. *Gammarus* sp. exhibits a distinct diel drift pattern, being found in larger numbers in night drift samples. *Gammarus pseudolimnaeus* has shown a similar pattern in a small stream (Waters 1962;

TABLE 3. Mean number of animals per treatment for the more common taxa collected in the Meldahl Dam experiment. Number in parentheses indicates number of samplers per treatment. Taxa which occurred in significantly greater numbers in either the fast-water ($F_5 + F_c$) or the slow-water ($S_5 + S_c$) conditions are indicated by an *F* or *S* in the column below such species. Number of asterisks indicates significance level (* = $P < 0.02$; ** = $P < 0.01$).

Treatment	*C. bicinctus*	*N. distinctus*	*P. convictum*	*P. illinoense*	*Rheotantarsus* sp.	*Gammarus* sp.	*H. orris*	*T. burksi*	Mean no. of animals per sampler
S_4 (2)	3.5	1.0	0.0	2.5	0.0	43.0	0.5	0.0	55.0
S_5 (4)	2.0	3.5	1.2	7.3	0.7	18.0	0.0	6.7	45.0
S_c (2)	1.5	3.5	1.5	4.0	1.0	13.0	0.0	9.5	41.0
$S-F_1$ (2)	0.0	0.5	0.0	3.0	1.5	9.5	0.5	1.0	24.0
$S-F_7$ (2)	1.5	2.5	2.5	1.0	1.0	3.0	2.0	2.5	19.0
F_4 (2)	7.5	2.0	5.0	0.0	3.5	1.0	16.5	2.0	50.5
F_5 (4)	5.5	6.2	5.5	0.3	13.5	0.5	8.7	2.0	58.0
F_c (2)	5.0	7.0	14.0	1.0	12.0	0.0	12.0	5.0	73.0
$F-S_1$ (2)	1.5	2.0	2.5	1.5	1.5	4.5	3.5	1.5	28.0
$F-S_7$ (2)	3.5	2.0	4.0	2.0	1.0	16.0	0.5	6.5	47.0
	F[a]**		*F***	*S***	*F***	*S***	*F***	*S**	

[a] *F* indicates this species is more abundant in fast-water conditions; *S* indicates greater abundances in slow-water conditions.

Holt and Waters 1967). In addition, Hughes (1970) and Wallace et al. (1975) showed in the laboratory that *Gammarus pulex* and *G. pseudolimnaeus*, respectively, were more active in slow currents than in fast rates of flow. It is probable, then, that substrates in slow waters in the Ohio R. were quickly colonized at night by active amphipods which were already numerous in such a habitat. The quicker rate at which *Gammarus* sp. colonized substrates transferred into slow water, in comparison to colonization rates by *H. orris* in fast water, was probably due to an active searching by amphipods in all directions in a slow current, while *H. orris* had to be "delivered" via drift along the direction of current onto a substrate "target."

The clear-cut difference in current velocity preferences exhibited by the congeneric larval chironomids *P. convictum* and *P. illinoense* (Tables 1 and 3) illustrates the importance of species level taxonomy and shows the potential errors inherent in interpreting environmental preferences or tolerances at the generic, rather than species, level. Resh and Unzicker (1975) pointed out the questionable value of the generic-level taxonomic unit as a water quality indicator. It is obvious from the *Polypedilum* data that even morphologically similar species can differ in preferences along an environmental gradient (current velocity in this case) and as with pollution tolerance should be approached from the species level.

The repetition in species abundance patterns exhibited over the various Ohio R. pools (Table 1) and subsequent experimental verification (at Meldahl Dam) of large-scale faunal dissimilarities in above-dam vs. below-dam macroinvertebrate communities point out the efficacy of current as a controlling factor in lotic systems. River discharge is equal to the cross-sectional area of the river × current velocity. In steady state conditions, therefore, as impounded rivers such as the Ohio increase in depth as they approach a dam (see Fig. 1) (and usually widen to some extent), cross-sectional area increases and current velocity concomitantly decreases. This study has investigated the two endpoints of invertebrate communities along this fast-to-slow current gradient. Community composition at any point between these two endpoints is a composite of individual species' responses to current (Gleason 1926; Whittaker 1975). Although current certainly also varies on a cross-river transect, our data suggest the existence of a general longitudinal (length of the river pool) zonation of macroinvertebrates as a function of gradual attenuation of current.

Acknowledgments

We thank Peter D. Spatt for his able help with the field portion of this study. We are grateful to the Meldahl Dam lockmaster, Mr Raymond Dunaway, and all the personnel at Meldahl Dam for their cooperation during the study. We also thank the U.S. Army Corps of Engineers for granting us permission to conduct the experiment at this site.

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Annual Production by Caddisflies and Mayflies in a Western Minnesota Plains Stream[1]

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MacFarlane, M. B., and T. F. Waters. 1982. Annual production by caddisflies and mayflies in a western Minnesota plains stream. Can. J. Fish. Aquat. Sci. 39: 1628 – 1635.

Annual production was estimated for five species of caddisflies and mayflies, comprising major components of the insect community, in the Redwood River, a second- to third-order plains stream in western Minnesota. Estmates were made at two sites, one above and one below an impoundment. At the upstream site, annual production ($g \cdot m^{-2}$, wet weight) and annual P/B ratios (in parentheses) were *Hydropsyche bifida*, 8.3 (6.9); *Cheumatopsyche pettiti*, 5.5 (7.0); *Stenonema nepotellum*, 3.4 (5.7); *Stenacron interpunctatum canadense*, 0.8 (7.0); and *Caenis simulans*, 4.7 (4.2); with total annual production of 22.7 $g \cdot m^{-2}$. At the downstream site, annual production and P/B ratios were *H. bifida*, 34.3 (4.4); *C. pettiti*, 68.5 (4.4); *S. interpunctatum canadense*, 24.1 (6.1); and *C. simulans*, 2.8 (4.4); with total annual production of 129.7 $g \cdot m^{-2}$. These species comprised 27.5% of the total insect standing stock at the upstream site and 75.9% downstream. The hydropsychid production at the downstream site was apparently sustained by the drift of zooplankton from the impoundment, resulting in considerably higher production by hydropsychids than reported in woodland streams.

Key words: production, Trichoptera, Ephemeroptera, plains stream, benthos, size–frequency method

MacFarlane, M. B., and T. F. Waters. 1982. Annual production by caddisflies and mayflies in a western Minnesota plains stream. Can. J. Fish. Aquat. Sci. 39: 1628 – 1635.

Nous avons estimé la production annuelle pour cinq espèces de phryganes et d'éphémères, principales composantes de la communauté d'insectes de la rivière Redwood, un cours d'eau de plaine de second à troisième ordre du Minnesota occidental. Ces estimations ont été faites à deux endroits, l'un en amont et l'autre en aval d'un réservoir de retenue. Au site d'amont, la production annuelle ($g \cdot m^{-2}$, poids humide) et les rapports P/B annuels (entre parenthèses) sont: *Hydropsyche bifida*, 8,3 (6,9); *Cheumatopsyche pettiti*, 5,5 (7,0); *Stenonema nepotellum*, 3,4 (5,7); *Stenacron interpunctatum canadense*, 0,8 (7,0); et *Caenis simulans*, 4,7 (4,2); avec production annuelle totale de 22,7 $g \cdot m^{-2}$. Au site d'aval, la production et les rapports P/B annuels sont: *H. bifida*, 34,3 (4,4); *C. pettiti*, 68,5 (4,4); *S. interpunctatum canadense*, 24,1 (6,1); et *C. simulans*, 2,8 (4,4); avec production annuelle totale de 129,7 $g \cdot m^{-2}$. Ces espèces représentent 27,5% de la biomasse totale d'insectes au site d'amont et 75,9% au site d'aval. La production d'hydropsychide au site d'amont semble dépendre de la dérive du zooplancton du réservoir de retenue, avec, comme résultat, une proportion de ces organismes beaucoup plus forte que celle mentionnée pour les cours d'eau de pays boisé.

Received February 25, 1982
Accepted July 26, 1982

Reçu le 25 février 1982
Accepté le 26 juillet 1982

There is growing interest among aquatic ecologists in the production biology of benthic invertebrates and in the use of production estimates to quantify energy flow through various components of aquatic ecosystems. Until recently, estimates of production by stream invertebrates have been scarce; however, at present, a sizable literature exists, having been published largely in the last decade (Waters 1977). Almost all data available have been obtained from small, low-order streams, particularly trout streams, where lack of turbidity, shallow depths, and easily sampled riffles apparently have facilitated bottom sampling. There are virtually no data available from the numerous prairie and plains streams of north

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Printed in Canada (J6819)
Imprimé au Canada (J6819)

central North America, many of which are affected by modern agricultural practices, characteristically have a high seston content from soil erosion, and are frequently turbid. Yet these often contain a rich benthic fauna dominated by immature insects classified as filterers or gatherers by Merritt and Cummins (1978), apparently using fine particulate organic matter (FPOM) as food.

Furthermore, there are still few data available for many quantitatively important taxa, for which methodological difficulties have been related to their type of life history. Earlier methods for production estimation have been generally limited to species with readily separated cohorts. However, the more recent size–frequency (Hynes) method does not require the separation of cohorts, thus circumventing this major problem (Hynes 1961; Hynes and Coleman 1968; Hamilton 1969; Benke 1979). Whereas the size–frequency method was originally proposed for gaining approximations of production by whole faunas or groups of combined species, most use of the method has been for single species, particularly for those that have extended periods of hatching and where separation of cohorts is difficult or impossible (Waters 1979). The diverse and widely distributed caddisfly family, Hydropsychidae, and several genera of mayflies fall into this category.

Many authors have conducted methodological studies, comparing the size–frequency method with earlier methods, almost always with similar results (Waters and Crawford 1973; Resh 1977; Cushman et al. 1978; Welton 1979), and the method has now been generally accepted as a valid procedure yielding accurate estimates (Benke and Waide 1977; Benke 1979; Waters 1979). Krueger and Martin (1980) have recently developed a method to compute confidence intervals about annual production estimates by the size–frequency method, and early applications of the method have resulted in remarkably good precision (Waters and Hokenstrom 1980).

The present study was conducted in the Redwood River, a western Minnesota plains stream, in which two widely distributed species of hydropsychid caddisflies and three species of mayflies constituted major components of the stream insect benthos. The study addressed two main objectives: first, to estimate production rates for these benthic insects common in a plains stream, and, second, to compare production between two communities, above and below an impoundment, having different quantities and qualities of particulate organic food resources.

Study Sites

The Redwood River is a tributary to the Minnesota River in southwestern Minnesota and is a plains stream now draining mostly agricultural cropland. Its watershed is divided into two distinct regions. From its headwaters in the southwest, the river crosses a gently rolling upland plain, the Coteau des Prairies, a prominent topographic feature cutting diagonally across southwestern Minnesota. The river drops from the Coteau into a lowland plain, also gently rolling but with lower relief, and continues in a northeasterly direction to the Minnesota River Valley. Before entering the Minnesota Valley, the Redwood flows through an impoundment, Lake Redwood, which formerly served a hydroelectric facility.

Sampling sites were selected from two quite different reaches of the river. An upstream site (Camden) was located on the slope off the Coteau in a wooded valley (second order). A downstream site (Ramsey) was located on the slope into the Minnesota River Valley, a short distance below Lake Redwood (third order). Both sites have coarse substrates and steep gradients, habitats not uncommon to plains streams (Jewell 1927), particularly where they fall over glacial lake beaches, the Coteau, or slopes into receiving streams.

Both sites had abundant populations of two species of hydropsychid caddisflies, *Cheumatopsyche pettiti* (Banks) and *Hydropsyche bifida* (Banks), as well as the mayflies *Stenonema nepotellum* (McDunnough) and *Stenacron interpunctatum canadense* (Walker) (both in Heptageniidae), and *Caenis simulans* McDunnough (Caenidae). The hydropsychids are classified trophically as filterers and all three mayflies as gatherers and/or scrapers (Merritt and Cummins 1978). As a group, these five species constituted an important component of the stream communities. While no work was conducted in the present study on the fishes of the Redwood River, or on their use of these insects as food, studies in other small streams in southwestern Minnesota indicated these and similar stream insects to be important in the diet of common stream fishes such as minnows, including the creek chub, blacknose dace, bigmouth shiner, and common shiner, and darters (Luey 1978).

Water temperatures ranged from 0°C in winter at both sites to ~22°C at Camden and 26°C at Ramsey in summer.

Methods

Seston Drift

Drift samples were collected to compare organic seston loads above and below the reservoir. Two nets as replicates were staked into the stream bottom, and samples were taken over 24-h periods on December 12–13, 1974, and June 17–18 and July 26–27, 1975.

Nets of 253-μm mesh were used for the December sampling, mainly as part of an invertebrate drift study, but the results are included here because of their relevance to seston drift. Nets of 100-μm mesh were used in the June and July sampling specifically for measuring seston. Material in all samples was screened through a 1000-μm mesh sieve to remove larger particles of detritus and macroinvertebrates. Thus, the remainder consisted of material between 253 and 1000 μm from the December samples and between 100 and 1000 μm from the June and July samples; the latter is a range that includes the mesh size of catch nets of most hydropsychids, at least in later instars (Wallace 1975; Wallace et al. 1977; Wallace and Merritt 1980). Small amounts of sand were removed by decantation. Organic material was dried to a constant weight at 60°C.

Benthic Invertebrates

A set of 10 samples of benthic invertebrates was collected from each of the two sites, at approximately monthly intervals from November 1974 through October 1975, using a 0.1-m^2 Surber sampler with a 471-μm mesh netting. Samples were preserved in the field with 10% Formalin and sorted in the

TABLE 1. Type of organic particles and percent composition (number) and total concentration (dry wt) (mean of two samples).

Date	Percent composition					Concn., $mg \cdot L^{-1}$
	Detritus	Copepoda	Cladocera	Rotifera	Eggs[a]	
			Camden			
Dec. 12–13, 1974	85.3	7.0	7.7	0.0	0.0	0.001
June 17–18, 1975	99.7	0.2	0.0	0.1	0.0	0.057
July 26–27, 1975	98.8	0.0	0.0	1.2	0.0	0.001
			Ramsey			
Dec. 12–13, 1974	3.9	10.4	85.7	0.0	0.0	0.012
June 17–18, 1975	27.5	0.1	1.2	20.3	50.9	0.102
July 26–27, 1975	99.9	0.1	0.0	0.0	0.0	0.027

[a]Small particles presumed to be eggs, but identity unknown.

laboratory. Mean wet weights were determined for each instar (hydropsychids) and for each size group (mayflies) by weighing a group of specimens after centrifuging in a screen cone to remove external liquids.

SPECIES IDENTIFICATIONS

Hydropsychid larvae and adults were identified using keys from Ross (1944) and later descriptions. Early instars were distinguished by head capsule width and coloration, using the methods of Mackay (1978). Head capsule widths were measured with an eyepiece micrometer, and the frequencies of these measurements were used to define a range in head capsule width for each instar of each species whereby the separate instars could be identified. *Hydropsyche bifida* larvae had slightly larger head capsule widths than *Cheumatopsyche pettiti* larvae in equivalent instars. Head capsule widths increased through instars by a factor of ~1.5 for both species as was true for the several species studied by Mackay (1978). All larvae from each month's collections were sorted into instars on the basis of the head capsule width ranges established for each species.

Mayfly nymphs were identified to genus with Edmunds et al. (1976) or Lewis (1974); subimagoes and imagoes were collected at the sampling sites at times of emergence or oviposition, identified to species with Burks (1953) or Lewis (1974), and presumed to correspond with nymphs as no other adults of the same genera were observed. Nymphs were sorted into size categories measuring head capsule widths of all specimens with an eyepiece micrometer.

PRODUCTION ESTIMATION

The size–frequency (Hynes) method was used for production estimation. Losses between successive size groups were multiplied by the number of size groups ("times loss" factor = *1* of Hamilton (1969) and Menzie (1980)) and summed algebraically for total annual production; however, negative values, when occurring in the early size groups, were considered to be zero, because these negative values were probably due to incomplete sampling of small specimens (Benke and Wallace 1980). The method of Krueger and Martin (1980) was used to calculate confidence intervals.

Benke's (1979) correction for cohort production interval (CPI) was used to account for periods in the life history when no aquatic production occurs, by multiplying by a ratio of 365/CPI in days. Based on data of Fremling (1960) and general ranges reported by Wiggins (1977), the egg stage of the hydropsychids was presumed to last about 15 d, the pupal stage about 7 d, and time between emergence and oviposition about 3 d. Thus a CPI of 340 d was used for estimates of production by univoltine hydropsychids. Hydropsychids were grouped into the five instars as size groups, and a "times loss" factor of 5 was used. Data reviewed by Edmunds et al. (1976) were used to estimate CPI for the mayflies. For the heptageniids, hatching was considered to occur about 20 d after oviposition, for *Caenis simulans* about 8 d. The time between emergence and oviposition was presumed negligible for all species. A CPI of 345 d was used for the heptageniids and 357 d for *C. simulans*.

Results

SESTON DRIFT

Drifting seston was consistently in higher concentrations at the Ramsey site (downstream from Lake Redwood) than at the Camden site (upstream), for all three dates, ranging from a low of 0.001 $mg \cdot L^{-1}$ at Camden (December and July) to a high of 0.102 $mg \cdot L^{-1}$ at Ramsey (June) (Table 1). The impoundment above Ramsey apparently was also responsible for greater proportions of animal materials in the seston, with higher amounts of Copepoda and Cladocera in December, and Rotifera and eggs (unknown identity) in June. Seston in late July was almost entirely of detritus at both sites. The concentrations reported here are low compared to other reported data, e.g. up to 8.2 $mg \cdot L^{-1}$ ash-free dry weight (AFDW) (Gurtz et al. 1980) and 3.68 $mg \cdot L^{-1}$ AFDW (Wallace et al. 1982); however, particles <100 μm (which frequently constitute the major portion of organic seston) were not collected in the present study.

LIFE HISTORIES

Life history patterns for all species were similar at the two sites. All appeared to be univoltine; however, there remained the possibility that *Cheumatopsyche pettiti* was bivoltine, es-

TABLE 2. Example of size–frequency calculation of annual production by *Cheumatopsyche pettiti* (wet wt).

Instar	$N \cdot m^{-2}$	Mean wt (mg)	Biomass ($g \cdot m^{-2}$)	$N \cdot m^{-2}$/ lost $\cdot m^{-2}$	Mean wt loss (mg)	Wt loss ($g \cdot m^{-2}$)	×5 ($g \cdot m^{-2}$)
			Camden				
I	90	0.13	0.01				
				−113	0.17	−0.02	0.00
II	203	0.24	0.05				
				11	0.39	0.00	0.00
III	192	0.61	0.12				
				31	1.15	0.04	0.20
IV	161	2.22	0.36				
				142	5.20	0.74	3.70
V	19	12.78	0.24				
				19	12.78	0.24	1.20
Total							5.10
Corrected annual production = 5.10 × 365/340 = 5.46							
			Ramsey				
I	31	0.13	0.01				
				−446	0.17	−0.08	0.00
II	477	0.24	0.11				
				−679	0.39	−0.26	0.00
III	1156	0.61	0.71				
				178	1.15	0.20	1.00
IV	978	2.22	2.17				
				−8	5.20	−0.04	−0.21
V	986	12.78	12.60				
				986	12.78	12.60	63.00
Total							63.79
Corrected annual production = 63.79 × 365/340 = 68.48							

pecially at the Ramsey site, where summer water temperatures were slightly higher. Pupation by hydropsychids began in May and extended through August or September. Hatching and recruitment of first instar larvae into the population began immediately in May or June and extended at least into September. Maximum overlap between cohorts occurred in June and July, and in the third and fourth instars, respectively. Larvae overwintered mainly in the second through fifth instars and a few in the first instar. No pupae were found during the winter.

The two heptageniid mayflies had emergence peaks in May or June, somewhat earlier than *Caenis simulans* which peaked in June and July. Maximum recruitment for all mayflies occurred shortly after the peak emergence, in June or July for the heptageniids and in July or August for *C. simulans*. All species had extended periods of recruitment and were broadly distributed among size groups throughout the entire year.

PRODUCTION

The size–frequency calculations of annual production by species are exemplified for a hydropsychid (*Cheumatopsyche pettiti*) in Table 2 and for a mayfly (*Caenis simulans*) in Table 3. The use of head capsule widths to place individual specimens into instars or size groups probably reduces the error mentioned by Waters and Crawford (1973) wherein the "times loss" factor may be inaccurately high because of the presence of one or a few unusual individuals in the largest size group when length is used to categorize groups.

Annual production by all five species totaled 22.7 $g \cdot m^{-2}$ (wet) at the Camden site and 129.7 $g \cdot m^{-2}$ at Ramsey (Table 4). The latter, below Lake Redwood, was nearly sixfold higher than at the upstream Camden site, probably reflecting the influence of the greater seston quantities below the impoundment. Total annual production by the two hydropsychids *H. bifida* and *C. pettiti* was 13.8 $g \cdot m^{-2}$ at Camden and 102.8 $g \cdot m^{-2}$ at Ramsey; for the mayflies, total annual production was 8.9 $g \cdot m^{-2}$ at Camden and 26.9 $g \cdot m^{-2}$ at Ramsey.

Annual production by individual species shows other differences between sites (Table 4). With the exception of the mayflies *C. simulans* and *Stenonema nepotellum*, all production rates were higher at the Ramsey site below Lake Redwood. Annual production by *C. simulans* was nearly twice as high at the upstream site, Camden (4.7 $g \cdot m^{-2}$), than at Ramsey (2.8 $g \cdot m^{-2}$), suggesting that this species was not benefiting from the greater seston at Ramsey, but rather that it was functioning more as a scraper than a gatherer in its trophic habits. A similar situation may apply for *S. nepotellum*; annual production was 3.4 $g \cdot m^{-2}$ at Camden, but negligible at Ramsey.

TABLE 3. Example of size–frequency calculation of annual production by *Caenis simulans* (wet wt).

Head width (μm)	$N \cdot m^{-2}$	Mean wt (mg)	Biomass ($g \cdot m^{-2}$)	$N \cdot m^{-2}$/ lost $\cdot m^{-2}$	Mean wt loss (mg)	Wt loss ($g \cdot m^{-2}$)	×10 ($g \cdot m^{-2}$)
			Camden				
0.38	13	0.34	0.004				
				−66	0.36	−0.024	0.00
0.39–0.50	79	0.42	0.033				
				−145	0.49	−0.071	0.00
0.51–0.62	224	0.57	0.128				
				−7	0.66	−0.005	0.00
0.63–0.74	231	0.76	0.176				
				1	0.86	0.001	0.01
0.75–0.86	230	0.98	0.225				
				42	1.16	0.049	0.49
0.87–0.98	188	1.38	0.259				
				129	1.66	0.214	2.14
0.99–1.10	59	2.00	0.118				
				39	2.49	0.097	0.97
1.11–1.22	20	3.11	0.062				
				6	3.74	0.022	0.22
1.23–1.34	14	4.49	0.063				
				6	5.09	0.031	0.31
1.35	8	5.78	0.046				
				8	5.78	0.046	0.46
Total							4.60
Corrected annual production = 4.60 × 365/357 = 4.69							
			Ramsey				
0.38	1	0.34	0.000				
				−40	0.36	−0.014	0.00
0.39–0.50	41	0.42	0.017				
				−96	0.49	−0.047	0.00
0.51–0.62	137	0.57	0.078				
				−5	0.66	−0.003	0.00
0.63–0.74	142	0.76	0.108				
				27	0.86	0.023	0.23
0.75–0.86	115	0.98	0.113				
				15	1.16	0.017	0.17
0.87–0.98	100	1.38	0.138				
				48	1.66	0.080	0.80
0.99–1.10	52	2.00	0.104				
				39	2.49	0.097	0.97
1.11–1.22	13	3.11	0.040				
				7	3.74	0.026	0.26
1.23–1.34	6	4.49	0.027				
				3	5.09	0.015	0.15
1.35	3	5.78	0.017				
				3	5.78	0.017	0.17
Total							2.75
Corrected annual production = 2.75 × 365/357 = 2.81							

Annual P/B ratios fell approximately in the range reported elsewhere for univoltine benthic invertebrates (Waters 1977), ranging from 4.2 to 7.0 (Table 4). For the hydropsychids, P/B ratios were higher at the Camden site (6.9–7.0) than at Ramsey (4.4). For the mayflies, no such difference between sites was found.

Discussion

Total hydropsychid production at Ramsey at 102.8 $g \cdot m^{-2}$ (wet) (~17.1 $g \cdot m^{-2}$ (dry)) is high relative to most hydropsychid estimates of production in the literature, although some are higher, e.g. 26.0 $g \cdot m^{-2}$ (dry) (Floessner 1976). Even higher production rates have been reported on the basis of snag surface area (Benke et al. 1979; Cudney and Wallace 1980).

Mayfly production generally was not high compared with others from the literature with the exception of the heptageniid *Stenacron interpunctatum canadense* at Ramsey. The highest

TABLE 4. Annual production (P, $g \cdot m^{-2}$, wet weight) and annual P/B ratios for insects in the Redwood River. Ninety-five percent confidence intervals are given in parentheses.

	Camden		Ramsey	
	P	P/B	P	P/B
Hydropsychidae				
Hydropsyche bifida	8.3 (7.47–9.11)	6.9	34.3 (31.88–36.72)	4.4
Cheumatopsyche pettiti	5.5 (4.65–6.27)	7.0	68.5 (64.96–72.00)	4.4
Total Hydropsychidae	13.8		102.8	
Ephemeroptera				
Stenonema nepotellum	3.4 (3.30–3.58)	5.7		
Stenacron interpunctatum canadense	0.8 (0.78–0.88)	7.0	24.1 (23.67–24.57)	6.1
Caenis simulans	4.7 (4.61–4.77)	4.2	2.8 (2.75–2.87)	4.4
Total Ephemeroptera	8.9		26.9	
Total	22.7		129.7	

production reported for Heptageniidae appears to be 2.08 $g \cdot m^{-2}$ (dry) for *Rhithrogena semicolorata* (Zelinka 1973). Although the estimate from Ramsey was considerably higher than this, it was still within the range for Ephemeroptera based on Waters's (1977) review and well below a recent estimate for *Tricorythodes atratus* (Hall et al. 1980). We are aware of no production estimates for species in the mayfly family Caenidae.

The five species selected for production estimates in this study represented 75.9% of the insect biomass at Ramsey and 27.5% at Camden, and of the total invertebrate biomass, 46.8% at Ramsey and 15.4% at Camden (calculated from data in MacFarlane 1978). Thus, total invertebrate production was much greater than the sum of these five insect species' production at either site, particularly at Camden. Two estimates of total insect production in streams are available for comparison, both from second-order, woodland streams. These estimates fall remarkably close together: 4.78 $g \cdot m^{-2}$ for Bear Brook (Fisher and Likens 1973); 4.80 and 4.32 $g \cdot m^{-2}$ (dry) for Factory Brook, 1974 and 1975 (Neves 1979). The five-species sum of production estimates at Camden, ~3.8 $g \cdot m^{-2}$ (dry), was near these values, and at Ramsey the sum was much greater, 21.6 $g \cdot m^{-2}$ (dry). Total insect production at both sites in this plains stream was apparently much greater than in the above woodland streams; this difference may be true for plains streams generally caused by abundant organic seston, particularly in regions of intensive agriculture where it apparently is derived from surrounding cropland soils.

Large production differences between the two sites were apparent. High hydropsychid production at Ramsey was presumably sustained by the higher sestonic drift at that site with its high proportion of zooplankton. High standing stocks of hydropsychids below lakes or reservoirs are common (Chutter 1963; Cushing 1963; Spence and Hynes 1971). The high proportion of animal food available in these habitats is a major factor; such animal food provides essential nutritional supplements to a diet mainly of detritus (Anderson 1976) and may account for the major share of hydropsychid production (Benke and Wallace 1980). Slightly coarser substrates at Ramsey presumably provided a greater quantity of optimal habitat for hydropsychids, also contributing to the difference.

Differences in mayfly production were more complicated but probably were also related to food and substrate combinations. Two species were more productive at Camden, *Stenonema nepotellum* and *Caenis simulans*. The coarser substrates at Ramsey provided abundant habitat for the heptageniids, and an abundant supply of FPOM was available in the sediments. *Stenacron interpunctatum canadense* was much more productive at Ramsey. *Stenonema nepotellum*, however, did not occur in significant numbers at Ramsey; perhaps this species and *C. simulans*, not benefiting from the larger quantities of FPOM at Ramsey, functioned more as scrapers in their trophic habits. Slightly finer substrates at Camden and some in-channel vegetation further improved the habitat for *C. simulans* at that site.

Confidence intervals calculated for these estimates ranged from 3.4 to 29.7% of production, seemingly low values when compared with the known high variance in stream bottom sampling. Krueger and Martin (1980) obtained intervals of 35.3–46.8% of production for their examples using data for two mayflies. Waters and Hokenstrom (1980) obtained smaller intervals, 11.1–15.6% of production by the amphipod *Gammarus pseudolimnaeus*, but took a larger number of samples (20) per day. Our confidence intervals were generally larger for the hydropsychids (10.3–29.7% of production) than for the mayflies (3.4–12.1% of production). Smaller confidence intervals were obtained for larger values of production by a given species. Spatial distributions appeared to exert an important influence on the size of the confidence interval. Although all species had contagious spatial distributions based on the Chi-squared variance to mean ratio (Elliott 1971), the mayflies appeared more evenly distributed than the hydropsychids, and the confidence intervals were smaller. Systematic errors, such as errors in determining CPI or voltinism, were probably small in view of the published data referred to in the Methods section.

Hydropsychid P/B ratios were substantially higher at Camden than at Ramsey (Table 4), but no comparable difference was apparent for the mayflies. Robertson (1979) in his review of marine P/B ratios points out that more older and larger individuals in a population reduces the P/B ratio, because these individuals increase mean population biomass but add relatively less to production. Hydropsychid survival into the last larval instar was much greater at Ramsey than at

Camden for both species and probably accounts for the higher P/B ratios at the Camden site (see data for *Cheumatopsyche pettiti* in Table 2; survival by *H. bifida* from IV to V instars was 158−22 m^{-2}, or 14%, at Camden, but was 357−344 m^{-2}, or 96%, at Ramsey). Greater survival at Ramsey may be due in part to more abundant space under cobbles at that site. At Camden, most cobbles were embedded in sand. At Ramsey, much of the larger interstitial space remained open; settling of coarser sands in the reservoir helps preserve these spaces where the larvae are better protected from scour during high water and from predation by fish. Greater survival to V instar at Ramsey might also be due to the greater abundance and higher quality of sestonic food at this downstream site.

The annual P/B ratios approximate cohort P/B ratios as all species were univoltine; however, cohort P/B ratios would in all cases be slightly lower, approximately in the proportion CPI/365, because the CPI for all species was slightly less than 365 d (Waters 1979).

Hynes (1980) cautions against the uncritical acceptance of confidence limits obtained from the method of Krueger and Martin (1980). Sampling problems, particularly on hard substrates, must keep us from feeling overly comfortable with narrow confidence limits; as studies of the hyporheic benthos clearly demonstrated (Coleman and Hynes 1970; Bishop 1973; Hynes 1974; Williams and Hynes 1974), systematic error in benthic sampling can be very large. However, as Benke et al. (1979) pointed out, samples from all dates through the year are dealt with as replicates in the size−frequency method, in effect increasing degrees of freedom and resulting in lower variance.

In this study, the smallest individuals of each species apparently were not sampled accurately, with the result being negative entries in the production tables for the first one or two size groups. These were treated as zero, as explained above, but this result still represents an error in the direction of an underestimate. The underrepresentation of smallest instars is probably due to several factors, including escapement through too large mesh in the sampler, rapid growth of early instars between sampling dates, and lack of detection in sample analysis because of their small size. The error is common in production studies and the degree of error unknown; however, in view of the extremely small size (i.e. weight) of the earliest instars, the error in terms of biomass may be small or even negligible.

The caddisfly and mayfly production estimates from the Redwood River provide data for a plains stream, a type of stream from which no previous production estimates have been available. Production apparently was influenced by the quantity and quality of organic seston loads, which probably were derived from cropland soils. Hydropsychids below the impoundment may have been particularly advantaged by the availability of animal food in the drift which increased their production.

Production by plains stream benthos appears high compared with available data on production in woodland streams. The high organic seston loads of plains streams, especially in regions of intensive agriculture, probably constitute a major factor increasing invertebrate production.

Acknowledgments

We are most grateful for assistance received from Dennis K. Shiozawa in field sampling and from Edwin F. Cook in identification of adult insects, and for aid in life history interpretation and review of the manuscript by Rosemary J. Mackay.

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Effects of Copper on Nitrogen Fixation and Growth of Blue-Green Algae in Natural Plankton Associations

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WURTSBAUGH, W. A., AND A. J. HORNE. 1982. Effects of copper on nitrogen fixation and growth of blue-green algae in natural plankton associations. Can. J. Fish. Aquat. Sci. 39: 1636–1641.

Copper toxicity bioassays were conducted on six stages of the spring *Aphanizomenon flos-aquae* bloom in eutrophic Clear Lake, California. Major variables tested were nitrogen fixation, carbon fixation, pigments, and cell numbers. Inhibition of nitrogen fixation, carbon fixation, and pigment accumulation increased linearly between 10 and 30 μg Cu/L. Higher concentrations produced little additional toxicity. In contrast, there was a stimulatory effect of copper, especially on nitrogen fixation, at the very low level of 2 μg/L. There was no inhibitory effect of copper on nitrogen fixation without a comparable or greater effect on carbon fixation or chlorophyll *a*. Other algae growing with the *Aphanizomenon* bloom were not affected by additions of copper up to 20–30 μg Cu/L, but were affected at the 50–100 μg/L level.

Key words: algae, algicide, blue-green algae, copper, cyanobacteria, metal, limiting nutrient, nitrogen fixation, photosynthesis, phytoplankton, toxicity

WURTSBAUGH, W. A., AND A. J. HORNE. 1982. Effects of copper on nitrogen fixation and growth of blue-green algae in natural plankton associations. Can. J. Fish. Aquat. Sci. 39: 1636–1641.

Des analyses biologiques de toxicité du cuivre ont été menées sur six étapes de la floraison printannière d'*Aphanizomenon flos-aquae* dans le lac Clear, un lac eutrophe de la Californie. Les principales variables analysées ont été la fixation de l'azote, celle du carbone, les pigments et le nombre de cellules. L'inhibition de la fixation de l'azote, de celle du carbone et de l'accumulation des pigments augmente de façon linéaire entre 10 et 30 μg Cu/L. De plus fortes concentrations produisent peu de toxicité additionnelle. Par contraste, le cuivre a une action stimulante, surtout sur la fixation de l'azote, aux niveaux très faibles de 2 μg/L. Le cuivre n'a pas d'effet inhibiteur sur la fixation de l'azote sans un effet comparable ou plus prononcé sur la fixation du carbone ou sur la chlorophylle *a*. D'autres algues croissant en même temps que la floraison d'*Aphanizomenon* ne sont pas influencées par l'addition de cuivre jusqu'à 20–30 μg Cu/L, mais le sont par 50–100 μg/L.

Received January 20, 1982
Accepted August 25, 1982

Reçu le 20 janvier 1982
Accepté le 25 août 1982

COPPER is used extensively as an algicide, particularly to control blue-green algae which are highly sensitive to this ion (Gibson 1972; Horne and Goldman 1974; McKnight 1981). Dosages between 50 and 2300 μg/L are commonly recommended to control algal blooms (U.S. Environmental Protection Agency, see Dunst et al. 1974), even though laboratory and some field tests have shown toxic effects in fresh water at levels below 25 μg/L (Steeman Nielson et al. 1969; Horne and Goldman 1974; Whitaker et al. 1978). Considering that maximum suggested copper pollution levels for protecting fish can be as low as 0.1 μg/L (Sauter et al. 1976), it is imperative that excessive doses of copper are not put into critical environments.

Copper toxicity is dependent on its speciation, chelated ions losing their toxicity (Sunda and Gillard 1976). However, Horne and Goldman (1974) demonstrated that copper additions as low as 5 μg/L can be toxic to natural lake algal populations, even though high levels of natural chelators were

Printed in Canada (J6790)
Imprimé au Canada (J6790)

presumably present. Their data indicated that low copper levels inhibited nitrogen fixation more than photosynthesis, thus suggesting why cyanophytes might be more sensitive to copper than other algal taxa.

The objective of our work was (1) to find minimal copper concentrations that could control blue-green algal blooms in a eutrophic lake; (2) to test the hypothesis of Horne and Goldman (1974) that nitrogen fixation in blue-green algae is highly sensitive to copper; and (3) to test the effects of natural and artificial chelators on toxicity. Because blue-green algae are notoriously unstable and frequently die before tests can be made, much of the available information on copper is based on laboratory cultures. We tried to overcome this problem by conducting bioassays frequently during the course of a large bloom of *Aphanizomenon flos-aquae* in Clear Lake, California.

Methods

Clear Lake, California (121°W, 39°N), is quite large (170 km^2), shallow (mean depth = 8.0 m), naturally eutrophic, warm, and polymictic. Mean chemical concentrations (mg/L) for the lake are alkalinity, 127; total hardness, 115; bicarbonate, 145; copper, 0.003 (Lallatin 1975; Horne and Goldman 1974). Unfortunately, pH values during the experiments are not available because of failure of our meter. However, typical values for this spring period and for similarly treated cultures in another set of experiments range from 7.5 to 8.7.

As a result of a large nutrient base, algal nitrogen fixation, and polymixis, blue-green algal blooms are frequent in Clear Lake and often reach nuisance proportions. The bioassays were conducted during the early portion of the spring algal bloom, which is dominated almost exclusively by *A. flos-aquae*. This bloom begins in April or May and usually peaks and collapses in June as a result of insufficient nitrogen and/or iron supplies (W. A. Wurtsbaugh and A. J. Horne unpublished data). Further details of the limnology of Clear Lake are in Goldman and Wetzel (1963) and Horne and Goldman (1972).

In vitro multiparameter bioassays were conducted on unconcentrated lake plankton following the methodology of Horne and Goldman (1974). Water samples for the bioassays were collected in the mornings from the pelagic area of Clear Lake with a plastic Van Dorn water bottle. Phytoplankton blooms change their physiology greatly and often irregularly during their course. To find which effects were of general applicability, and which were specific to certain stages, we made many assays through the course of the bloom. A series of 4-L acid-washed Erlenmeyer flasks (Pyrex) were each filled with 2 L of lake water and closed with a cotton plug. The flasks were incubated at room temperature (17–22°C) under continuous fluorescent light (General Electric cool white bulbs) at intensities of 950–1250 lx (~40 $\mu Einsteins \cdot m^{-2} \cdot s^{-1}$). The flasks were swirled gently by hand for several seconds twice daily to redistribute the algae, and randomly redistributed in the incubation chamber to minimize position effects.

Treatments receiving copper were duplicated and two or three untreated flasks were used as controls. Copper solutions were made from reagent grade salts of $CuSO_4 \cdot 5H_2O$. The effect of the metal chelator disodium (ethylenedinitrilo)tetraacetate (EDTA) on copper toxicity was tested in several experiments. In one experiment copper was mixed with 1 cm of Clear Lake sediment in 65 mL of water, allowed to stand for 1 h, filtered through a Whatman GFC filter, and the filtrate then added to the appropriate flasks. A control received the filtrate from a sediment–water mixture without added copper.

Each of the six bioassays lasted 5–7 d. At 1- or 2-d intervals, duplicate subsamples from each flask were taken for measurements of nitrogen and carbon fixation. Single samples were taken for analysis of chlorophyll *a*. Chlorophyll was measured spectrophotometrically after extraction with 90% aqueous methanol because acetone is a poor solvent for blue-green algal chlorophyll (Talling and Driver 1963; Rai 1980). Carbon fixation was measured using the ^{14}C technique as described by Goldman (1963). Nitrogen fixation was measured by the acetylene reduction method (Stewart et al. 1967) as described by Horne and Goldman (1972). The subsamples for nitrogen and carbon fixation were incubated for 2 h under the same temperature and light conditions as the culture flasks. Biomass estimates of algal taxa were made on subsamples preserved in Lugol's solution. Counting was done in a Sedgwick–Rafter cell and ~9% of the cell volume was counted. Algal volumes were estimated by assuming that the algae conformed to fixed geometric shapes. The resuls from each day were analyzed by one-way analysis of variance and each treatment compared with the control by linear contrasts using a program provided by Tektronix (1974).

Results

Six bioassay experiments were performed during the 1973 bloom. For brevity, the results of only one experiment (II) are shown in detail (Fig. 1). Full details of the other experiments (Table 1) are available from the authors. Experiment II was most complete in terms of the number of variables measured, and the control cultures grew actively and maintained moderately high nitrogen fixation rates throughout the test.

In Experiment II copper toxicity was pronounced at levels of 20 μg/L. Toxicity increased at 30 μg/L, but at 75 μg Cu/L little additional inhibition was observed (Fig. 1). Carbon fixation was the most sensitive parameter measured, with significant inhibition at 10 μg/L, and perhaps at 5 μg Cu/L (Fig. 1C). At copper levels of 30 μg/L and above, carbon fixation was reduced to 10–20% of controls within 2 d, and remained at those levels throughout the experiment. Nitrogen fixation rates were significantly affected only by copper additions of 20 μg/L or more (Fig. 1B). At this concentration, nitrogen fixation dropped to 45% of controls within 1 d and remained at this level throughout the experiment. At 30 and 75 μg/L, nitrogen fixation rates fell essentially to zero within 48 h. Chlorophyll was not markedly influenced by copper additions of 5 or 10 μg/L, but chlorophyll loss was nearly complete at 30 or 75 μg/L (Fig. 1A). Chlorophyll concentrations responded to copper additions more slowly than did carbon or nitrogen fixation. At most copper levels, inhibition was not complete until 72–96 h (Fig. 1A). This may, in part, be an artifact of our assay technique, because we did not

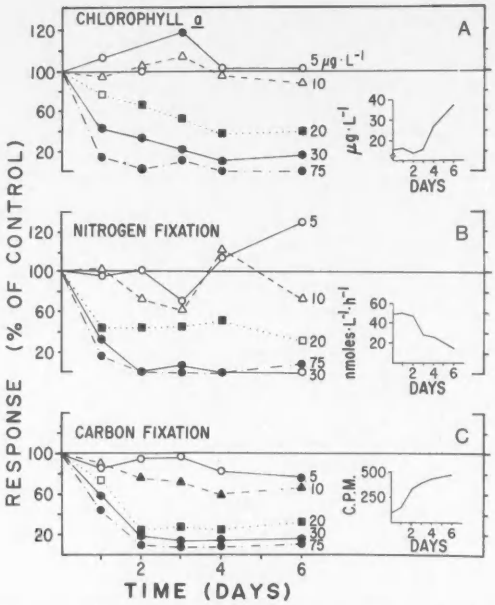

FIG. 1. Effects of copper on chlorophyll *a* (A), nitrogen fixation (B), and carbon fixation (C), of a natural plankton association from Clear Lake, California (Experiment II; April 18–24, 1973). Results are plotted as percentage of control except in the small inserted graphs which indicate absolute changes in the control flasks. Nitrogen fixation rates expressed as moles of acetylene reduced. Numbers by each line show added copper concentrations in μg/L. Solid symbols indicate a treatment response significantly different from controls ($P < 0.05$).

correct for chlorophyll degradation products. These pigments may have remained in the cultures for some time after the chlorophyll molecules lost their structure. Additionally, leaching of chlorophyll from dead or dying cells may have caused a lag in the decline of pigments.

Biomass estimates made after 6 d exposure to copper indicated that the blue-green alga *A. flos-aquae* was more sensitive to copper than were diatoms (Fig. 2). Final mean volumes of *Aphanizomenon* were reduced by any addition of copper, but treatments were not significantly different ($P < 0.05$) from controls below 20 μg/L. At 30 μg Cu/L *Aphanizomenon* volumes were reduced to 20% of control levels and at 75 μg/L were only 1% of controls. In contrast, diatom volumes at 30 and 75 μg Cu/L were 35 and 20% of control levels, respectively. Even these reductions were not significantly different from controls, due, in part, to the high variability in the 10 μg Cu/L treatment. If this unexplainably high variance was removed from the analysis of variance, diatom volumes were significantly different from controls at both 30 and 75 μg Cu/L. Small numbers of flagellates also present in the cultures showed no significant response to copper at all levels tested.

Results in the other experiments (Table 1) were similar. In all of the experiments, *Aphanizomenon* represented over 99% of the initial algae biomass present (Table 1). In two of the experiments (III and IV) chlorophyll levels declined steadily throughout the experiment, indicating that the algae were stressed by some factor. Nitrogen fixation rates also dropped quickly to zero in control cultures of two experiments (IV and V) and treatment responses are consequently not included. In spite of the poor condition of the lake phytoplankton used in some experiments, the results are relatively consistent between experiments, except that the algae were less sensitive to copper in April than they were in May. In April (Experiments I–III), carbon and nitrogen fixation and chlorophyll were hardly affected at copper concentrations of 10 μg/L, although in May this concentration caused a 45–80% inhibition of these parameters (Table 1).

The pooled data from all of the bioassays indicate that the responses of nitrogen fixation, carbon fixation, and chlorophyll to copper addition were similar (Fig. 3). A 50% inhibition of all three parameters occurs at 15–20 μg/L of added copper, and inhibition is nearly complete at 30 μg/L. It appears that copper levels below 30 μg/L affect nitrogen fixation less than carbon fixation and chlorophyll levels (Fig. 3). Indeed, copper actually stimulates nitrogen fixation at levels near 2 μg/L. Stimulation of nitrogen fixation by 2 μg Cu/L occurred in both experiments where it was successfully assayed, but results were significantly above control levels on only 1 d (Table 1). At copper concentrations above 30 μg/L, carbon fixation rates appeared to be the least affected parameter (Fig. 3). This was probably an artifact of our method. Carbon fixation was normally measured without correction for dark-bottle uptake. In Experiment I, dark uptake of carbon was measured in some treatments and found to be 15–20% of light uptake, even at high copper concentrations. Consequently, our procedure would indicate significant uptake, even when photosynthesis had dropped to zero.

There was no significant correlation between initial cell volumes in our cultures and the estimated copper concentrations which caused a 50% reduction in ^{14}C uptake ($r = 0.35$; $0.50 < P < 0.20$; Table 1), suggesting that algal densities, in the range studied, may not affect toxicity. This agrees with the field results of Gächter (1976) but contrasts with the results of Steeman Nielson et al. (1969) and Gächter (1976), who found that increasing concentrations of algae in artificial cultures decreased copper toxicity.

The chelator EDTA was effective in inhibiting copper toxicity, but only if sufficient time was allowed for the copper–ligand reaction to occur before the chelate was added to the cultures. If copper was added to the cultures 3 min before EDTA, most of the toxic action was still evident (Fig. 4A). However, if the EDTA and copper were mixed for 1–3 min before addition to the cultures, most or all of the toxicity was removed (Fig. 4B–D). Toxicity was reduced ~40% when copper was mixed with lake sediments (Fig. 4D).

Discussion

Horne and Goldman (1974), working with natural associations of blue-green algae, found that copper inhibited nitrogen fixation more than either carbon fixation or chlorophyll *a* accumulation. Our work contrasts strongly with this result, as

TABLE 1. Effects of copper additions on nitrogen fixation, carbon fixation, and chlorophyll *a* levels in the six bioassays. Initial algal volumes and growth (mean % daily change in chlorophyll *a*) of control cultures is also indicated for each experiment. The response of the cultures are given as means of percentage of controls throughout the experiment; a treatment which was 20% below controls on 2 days and 60% below controls for 2 additional days would have a relative response of 40% of controls. Values in boldface type indicate treatments which were significantly different from controls ($P < 0.05$) on 2 or more days of the experiment. Values in italics were significantly different on only 1 day.

Exp. No. and date	Initial algal volumes[a] ($mm^3 \cdot L^{-1}$)	Control growth ($\% \cdot d^{-1}$)	Bioassay	Relative response (% of control)						
				Copper added ($\mu g \cdot mL^{-1}$)						
				2	5	10	20	30	75	100
I	Aph. 20.700	27	C Fix	105	—	*80*	—	**25**	—	**15**
April 11–16	Flag. 0.260		N_2 Fix	105	—	100	—	**10**	—	**0**
			Chl. *a*	125	—	100	—	**30**	—	**0**
II	Aph. 11.800	37	C Fix		*85*	**75**	**40**	**25**	**15**	
April 18–24	Diat. 0.004		N_2 Fix		105	85	**45**	**15**	**5**	
	Flag. 0.800		Chl. *a*		110	**100**	**55**	**25**	**5**	
III	Aph. 26.290	−14	C Fix		**125**	100	**30**	**20**	**20**	
Apr. 30–May 5	Diat. 0.006		N_2 Fix		110	100	**45**	**20**	**20**	
	Flag. 0.005		Chl. *a*		110	*85*	**25**	**10**	**0**	
IV	Aph. 13.990	−18	C Fix		**65**	**50**	**40**	**45**		
May 9–14	Diat. 0.002		N_2 Fix		—	—	—	—		
			Chl. *a*		**60**	**20**	**10**	**10**		
V	Aph. 10.740	22	C Fix	100	**75**	**45**	**40**	**40**		
May 16–20	Diat. 0.010		N_2 Fix	—	—	—	—	—		
	Flag. 0.003		Chl. *a*	110	**60**	**35**	**25**	**15**		
VI	Aph. 21.590	27	C Fix	*85*	**75**	**55**				
May 17–22	Diat. 0.006		N_2 Fix	*125*	*95*	**35**				
	Col. C 0.142		Chl. *a*	*85*	*80*	**55**				

[a] Aph. = *Aphanizomenon*; flag. = flagellates; Diat. = pennate diatoms; Col. C. = colonial ciliates; C Fix = carbon fixation; N_2 Fix = nitrogen fixation; Chl. *a* = chlorophyll *a*.

copper had a similar (or perhaps weaker) effect on nitrogen fixation than on carbon fixation or chlorophyll (Fig. 3). The spring bloom we tested consisted almost exclusively of *Aphanizomenon*. In contrast, Horne and Goldman tested a fall algal bloom in Clear Lake which is composed not only of nitrogen-fixing blue-green algae (*Aphanizomenon* and *Anabaena*), but also a sizable component of green algae and diatoms. At least some green algae and diatoms are relatively insensitive to copper (see below). Consequently, in a mixed population, the effects of copper on overall photosynthesis — attributable to blue-greens plus other algae — would be less than on nitrogen fixation which is attributable to blue-greens alone. Thus there is no reason to believe that copper is more toxic to nitrogen fixation than to carbon fixation in blue-green algae. It is possible that the decline in nitrogen fixation observed after copper treatments is indirectly caused by the breakdown of carbon fixation in vegetative cells which channel energy to the heterocyst. If the energy supply in the heterocyst is insufficient to remove oxygen, irreversible inactivation of the nitrogenase enzyme and nitrogen fixation could occur (Fay and Cox 1967; Murray and Horne 1980).

The *Aphanizomenon flos-aquae* we tested, and the results of other investigations (Fitzgerald and Faust 1963; Gibson 1972; Horne and Goldman 1974; Goldman et al. 1975; Altenhofen 1978; Elder and Horne 1978), indicate that some species of blue-green algae are more sensitive to copper than are other algal taxa. As nitrogen fixation is not especially sensitive to copper, we cannot evoke this explanation to explain the higher sensitivity of blue-green algae. Gibson (1972) found that the blue-green alga *Anabaena flos-aquae* binds greater quantities of copper than the green alga *Scenedesmus quadricauda* and that the toxicity per unit of copper uptake appeared similar in the two species. The reason for the greater uptake of copper by *Anabaena* is unknown.

We suggest that the question of relative toxicity of different algae may best be pursued at lower taxonomic levels than phyla. The early work of Maloney and Palmer (1956) showed nearly as much variance within phyla as between phyla, and the sensitivity of the marine blue-green alga *Oscillatoria thiebautii* to cupric ions (Rueter et al. 1979) is within the range found for marine diatoms (Sunda and Gillard 1976; Morel et al. 1978). Consequently, at the level of phyla there appears to be no reason to conclude that all blue-green algae are more sensitive to copper than are other taxa.

Copper added to our cultures in the range of 2–5 μg/L stimulated metabolism in several instances (Table 1; Fig. 3). Although this stimulation was not great, and usually not statistically significant, the positive effect of low copper levels on the *Aphanizomenon* blooms in Clear Lake has subsequently been supported by field experiments (Williams and

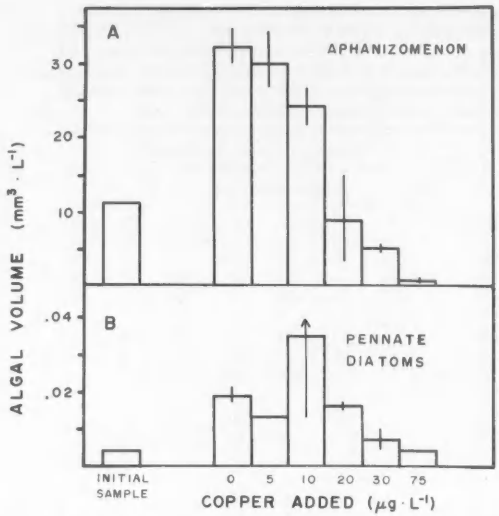

FIG. 2. Effects of copper additions on biomasses of Clear Lake algae in Experiment II. The volume of each taxon at the start of the experiment and after 6 d exposure to various copper levels is shown. Error bars, when greater than the width of the line, indicate the range of the duplicate cultures. A. *Aphanizomenon flos-aquae*. B. Pennate diatoms. Note different scales for each taxon.

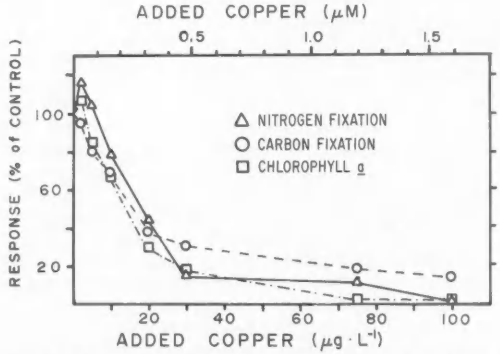

FIG. 3. Summarized results of the effects of copper on nitrogen fixation, carbon fixation, and chlorophyll *a*. Plotted values are mean responses for the six bioassays conducted during a spring *Aphanizomenon flos-aquae* bloom in Clear Lake. Some values for carbon fixation may be erroneously high (see results).

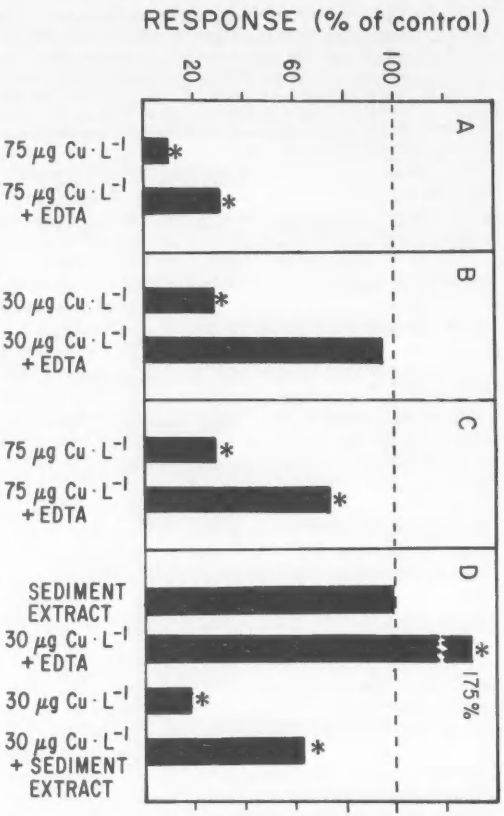

FIG. 4. Effects of chelators on copper toxicity. Responses are plotted as percent of control carbon fixation rates after 48-h exposures. A. EDTA was added to the cultures 3 min after copper was added (Experiment II). B. The EDTA was mixed with copper for 2 min before addition to the cultures (Experiment V). C. The EDTA was mixed with copper for 1 min before addition to the cultures (Experiment III). D. Copper was mixed with EDTA for 3 min, or with lake sediment for 60 min before addition to the cultures (Experiment IV). EDTA was always added in excess at a concentration of 1.3×10^{-5} *M*. Treatments marked with asterisks were significantly different from control cultures ($P < 0.05$).

Horne unpublished data; Goldman et al. 1975). It is possible that the stimulation we observed was caused by chemical changes in the media due to the copper additions or to toxic effects on bacteria (Gadd and Griffiths 1978) which could alter competitive relationships. However, algae do require copper for growth (Walker 1953; Manahan and Smith 1973), and copper levels in Clear Lake are generally low. Much of the copper in solution in Clear Lake may be unavailable, as a result of chelation by dissolved organic matter and by siderochromes excreted by blue-green algae (McKnight and Morel 1980). Telitchenko et al. (1970) reported a positive correlation of a blue-green algal bloom with copper levels, and Rueter et al. (1979) found a stimulatory effect of copper additions on some collections of *O. theibautii*. Thus it appears that low level additions of copper can stimulate algal growth in a variety of systems.

Our data indicate that between 30 and 75 μg Cu/L will effectively suppress *Aphanizomenon* blooms in Clear Lake. However, dosages as low as 5 μg Cu/L may be effective at certain times (Horne and Goldman 1974). A lake's chelation capacity may be one of the most important factors controlling treatment efficacy (Elder and Horne 1978). However, the rate

at which chelation occurs is also important, as algal inhibition by copper can be effected within minutes (Fig. 4; Elder and Horne 1978; Anderson and Morel 1978). Detoxification of copper by sediments (Fig. 4; Lewis et al. 1973) due to adsorption, chelation, or other modifications could also alter efficacy, especially in small shallow lakes with high basin area to volume ratios. Unfortunately, natural factors controlling chelation and detoxification by sediments are poorly understood and consequently copper dosages necessary for control cannot be reliably predicted. Prior to lake treatments, short-term bioassays of 1- to 2-d duration could be used in lieu of more detailed information to estimate minimal effective doses of the algicide. Minimal dosages should be sought in all cases, not only to minimize costs, but also to protect other aquatic biota.

Acknowledgments

Coen Carmiggelt and Krel Bradley assisted in various aspects of the study and John Elder and Diane McKnight critically reviewed early versions of the manuscript. This work was supported by the Clear Lake Flood Control and Water Conservation District and the California Department of Water Resources.

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The Recent Introduction of the Seagrass *Zostera japonica* Aschers. and Graebn. to the Pacific Coast of North America

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HARRISON, P. G., AND R. E. BIGLEY. 1982. The recent introduction of the seagrass *Zostera japonica* Aschers. and Graebn. to the Pacific coast of North America. Can. J. Fish. Aquat. Sci. 39: 1642 – 1648.

The seagrass *Zostera japonica* Aschers. and Graebn., recently introduced to British Columbia, Washington, and Oregon, is smaller than the native eelgrass *Z. marina* L.; grows intertidally where *Z. marina* is rare; and is an annual, overwintering as buried seeds, or a short-lived perennial, whereas the longer-lived perennial *Z. marina* usually overwinters as leafy shoots. Establishment of new populations of *Z. japonica* occurs mainly by seed germination although the chance of a seedling surviving its first summer is small. Once established, patches expand in area exponentially in spring and may help to stabilize the sediment. The fact that several areas where *Z. japonica* is abundant are or were sites of intensive oyster cultivation suggests that the plant was introduced as a contaminant (probably seeds) in shipments of Japanese oysters in the first few decades of the 20th century. Now common on the central coast of Washington, in northern Puget Sound, and in the southern Strait of Georgia, *Z. japonica* occurs as far south as Coos Bay, Oregon, and has the potential to spread further north and south.

Key words: biogeography, distribution, establishment, introduction, oyster culture, seagrass, *Zostera americana*, *Zostera japonica*

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Zostera japonica Aschers. et Graebn., herbe marine récemment introduite en Colombie-Britannique et dans les États de Washington et d'Orégon, est plus petite que l'herbe à bernaches indigène, *Zostera marina* L.; elle croît dans la zone intertidale, là où *Z. marina* est rare; et enfin c'est une plante annuelle hivernant sous forme de graines enfouies, ou encore une plante vivace à courte durée de vie, alors que *Z. marina*, qui est une plante vivace à plus longue durée de vie, hiverne ordinairement comme pousses feuillues. Les nouvelles populations de *Z. japonica* s'établissent ordinairement par germination des graines, bien que les jeunes plants aient peu de chance de survivre à leur premier été. Une fois établis, les lits s'étendent de façon exponentielle au printemps et peuvent contribuer à stabiliser le sédiment. Le fait que plusieurs régions où *Z. japonica* est abondante sont présentement, où étaient jadis, des sites d'ostréiculture intensive laisse supposer que la plante a été introduite comme contaminant (probablement en graines) en même temps que l'huître japonaise dans les quelques premières décennies du 20[e] siècle. Maintenant commune sur la côte centrale de Washington, dans le nord du Puget Sound et dans le sud du détroit de Géorgie, *Z. japonica* se rencontre aussi au sud que la baie Coos, en Orégon, et peut s'étendre encore davantage, tant au nord qu'au sud.

Received May 11, 1982
Accepted August 27, 1982

Reçu le 11 mai 1982
Accepté le 27 août 1982

THE rooted flowering plants called seagrasses are the most conspicuous vegetation in shallow-water, soft-bottom habitats on Canada's Pacific and Atlantic coasts. Eelgrass (*Zostera marina* L.) forms dense beds of leafy shoots year-round on lower intertidal and subtidal sand and mud, providing many crustaceans, fish, and birds with protection and food. Among the commercially important animals which use eelgrass beds are Dungeness crabs (*Cancer magister*) for protection and indirectly for food (Spencer 1932), and Pacific herring (*Clupea harengus pallasi*) as adults for attachment of eggs (Taylor 1964). Recently another seagrass, with a different life history, morphology, and preferred habitat, has invaded southwestern British Columbia. The purpose of this report is to acquaint marine biologists with the seagrass *Zostera japonica* Aschers. and Graebn., its taxonomic position, life

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Printed in Canada (J6929)
Imprimé au Canada (J6929)

history, present distribution in North America, introduction to the area and subsequent spread, and rates of establishment and growth.

Taxonomy of *Zostera japonica*

Zostera japonica has been recognized as a taxon distinct from the native eelgrass (*Z. marina*) since its first recorded collection on the Pacific coast of Washington state in 1957 (Hitchcock et al. 1969), but it has been assigned several specific epithets. Hitchcock et al. (1969) called it *Zostera nana* Roth, a taxon which included the European and Asian species of the subgenus *Zosterella* which are now called *Z. noltii* Hornem. and *Z. japonica*, respectively. In his monograph on seagrasses, den Hartog (1970) discarded the taxon *Z. nana* and recognized several species based on morphology of the leaf tip and the retinacula (sterile flaps of tissue in the inflorescences; Fig. 1B). The plants from Washington state were described as the new species *Zostera americana* den Hartog (Fig. 1A).

Comparisons of local material with collections and/or den Hartog's drawings of the European and Asian species led to the suggestions that *Z. americana* was really *Z. noltii* (Phillips and Shaw 1976) or *Z. japonica* (Harrison 1976), but neither of those studies was thorough enough to warrant a taxonomic revision. When a detailed morphological and anatomical comparison of the three taxa was made, using principal components analysis, *Z. noltii* was clearly distinct while *Z. japonica* and *Z. americana* had overlapping features and could not be separated (Bigley 1981).

Often *Z. marina* in intertidal beds is stunted and superficially resembles *Z. japonica*. The two can be distinguished on the basis of the leaf sheath which is a complete tube encircling the inner leaves in *Z. marina* but consists of two overlapping flaps in *Z. japonica* (den Hartog 1970; Fig. 1C).

Life History

Zostera japonica overwinters as buried seeds which germinate in spring (March to May in British Columbia). Vegetative growth produces branched rhizomes (underground stems) up to 50 cm long with nodes at intervals of 2–10 cm from which arise short roots and erect, leafy shoots 5–50 cm long (typically about 20 cm). Many shoots branch and produce flowers in summer (Fig. 1A). Seeds are shed in early autumn and most shoots die before November. In habitats sheltered from storm damage, especially in the low intertidal zone, short vegetative shoots overwinter, but many of those shoots die the next spring. Details of life history can be obtained from Miki (1933), Arasaki (1950a, b), Harrison (1979, 1982a, c), and Bigley (1981).

Present Distribution

Zostera japonica has been observed by us or reported by others from many localities between Coos Bay, Oregon, and Vancouver, British Columbia (Fig. 2). Most reports are from two centers, one in Willapa Bay and Gray's Harbor on the outer coast of Washington State and the other in northern Puget Sound (Washington) and the adjacent southern coast of the British Columbia mainland.

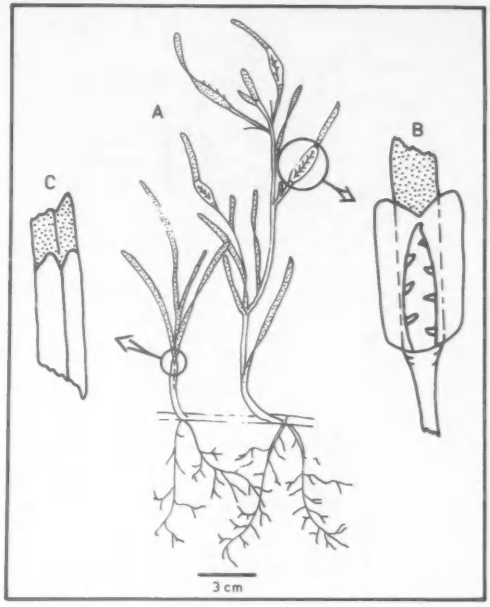

FIG. 1. *Zostera japonica*. A. Whole plant, showing vegetative shoot (left), branched flowering shoot (right), horizontal rhizome, and roots. B. Detail of inflorescence with flowers removed and spathe opened to show, in this specimen, seven retinacula. C. Detail of leaf sheath (unshaded), showing the two overlapping flaps.

In the first center, the most extensive *Z. japonica* beds in North America are found on the 17 000 ha of intertidal flats of Willapa Bay where all substrates except those with excessive clay or gravel support dense populations. Gray's Harbor also has some large beds, but near the industrial centers of Aberdeen and Hoquiam the plant is absent from many sites which have apparently suitable intertidal substrates. Perhaps the presence of waterborne pollutants is limiting the growth of *Z. japonica*, as was suggested by Miki (1933) in Japan.

In the second center, the largest populations of *Z. japonica* occur on the muddy and sandy shores of Padilla and Samish bays in northern Puget Sound and the Birch–Semiahmoo–Boundary Bay system flanking the Canada–USA border. Between those two areas (in Bellingham and Chuckanut bays) and to the north of the border on Roberts and Sturgeon banks and in the Nanaimo estuary (Vancouver Island) are what we believe to be recently established populations; they consist of small patches but are spreading rapidly (see section on Establishment and Growth). The plants in Hood Canal, Washington (Fig. 2), are probably the descendents of transplants from Willapa Bay (R. C. Phillips, Seattle Pacific University, Seattle, Washington, 1982, personal communication).

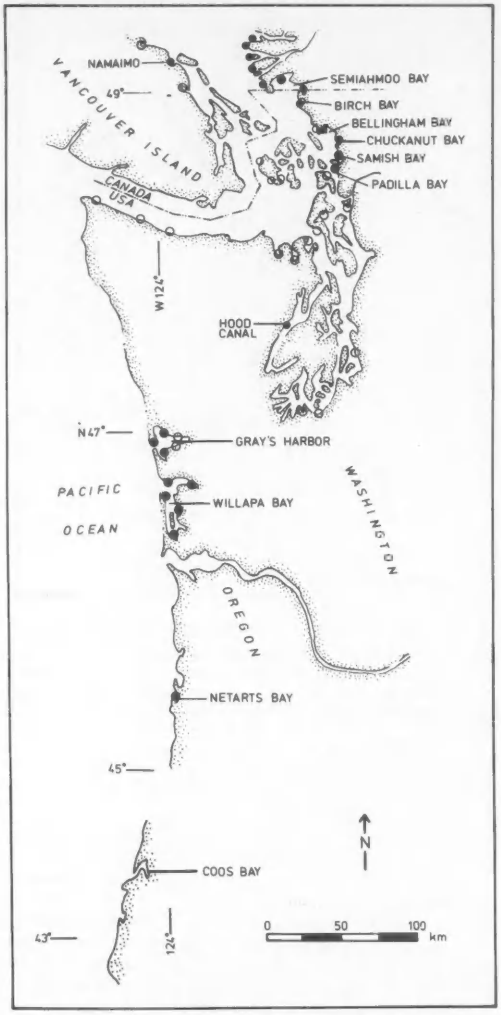

FIG. 2. Distribution of *Zostera japonica* in British Columbia (Canada) and Washington and Oregon (USA). Closed circles = sites where plant has been found. Open circles = sites where substrate is apparently suitable, but plant was absent in 1980–81.

Introduction and Spread of *Z. japonica*

As with most cases of inadvertent introduction, documentation of the time and method is difficult, but at least two lines of evidence point to the recent (i.e. 20th century) introduction of *Z. japonica*. First, the marine flora of the area has been studied extensively since the late 1800s (see summary in Scagel 1957), and whereas many subtidal species have been discovered recently, it is unlikely that the present extensive intertidal beds of *Z. japonica* would have been overlooked on earlier surveys. Second, the speed with which the plant's distribution is expanding (Fig. 3A) suggests either a recent introduction or a modification of the habitat, and because no large changes in sediment type or its deposition patterns have been documented in the area (Kellerhals and Murray 1969; Swinbanks 1979), we believe the former. On a small scale, however, deposition of fine sediment has been favored by the construction of causeways and breakwaters on the Fraser foreshore (Luternauer 1980; Harrison 1981), and the change may be the reason that the plant is spreading in that area. Thus the first collection in 1957 sets the latest time of introduction although the actual date may have been decades earlier.

Consideration of the possible mode of introduction of the Asian seagrass may lead to a clearer idea of the time of the event. The two main areas of *Z. japonica* in North America, Willapa Bay and northern Puget Sound, were the sites of the first cultivation of Japanese oysters (*Crassostrea gigas* Thunberg) on the west coast. In 1902 the failing commercial success of the native oyster (*Ostrea lurida* Carpenter) and the high cost of importing oysters (*Crassostrea virginica* Gmelin) from the Atlantic coast brought on the first large-scale introduction of Japanese oysters from Akkeshi Bay, Hokkaido (Japan), to Samish Bay in Puget Sound (Hori 1947). For decades "seed" oysters were imported almost every year to Samish Bay, but not until 1928 did the first small shipment arrive in Willapa Bay. The success of that transplant led to increasing numbers of oysters being introduced in the following years (Holway 1934; Sayce 1976).

Several animal species including the Japanese oyster drill (*Ocenebra japonica*) and *Batillaria zonalis* are thought to have arrived within the cases of Japanese oysters (Quayle 1964). Also, Scagel (1956) proposed that the alga *Sargassum muticum* (Yendo) Fensholt was introduced to the west coast in the same manner. Several parallels exist between the early records of *S. muticum* in oyster-growing areas and the first records of *Z. japonica*.

Although the importation of oysters continues, precautions against accidental introductions of other organisms have been in place since the early 1950s (Quayle 1953). Thus contaminants most likely would have arrived in the 1930s and early 1940s when large oyster shipments occurred with minimal precautions. It is easy to imagine that a few dormant seeds could have survived the journey among the shells and then germinated when returned to a suitable habitat. Alternatively, it has been suggested (as reported by Harrison 1976) that oysters were packed in seagrass, so shoots (vegetative or seed-bearing) may have been introduced when the crates of oysters were unpacked.

Because of the widely separated centers of distribution in North America and ample opportunity for transport through oyster shipments, we think that *Z. japonica* may have been introduced on a number of occasions. By 1974–75 it was common not only on the Pacific coast of Washington State but also in northern Puget Sound and in Boundary Bay (Phillips and Shaw 1976; Fig. 3A). By 1978 it had spread 25 km northwestward along the Fraser River foreshore and in 1979 it reached Vancouver Island, 35 km across the Strait of Georgia from the nearest colonized site on the mainland (Fig. 3A). The mode of local dispersal is uncertain. Both

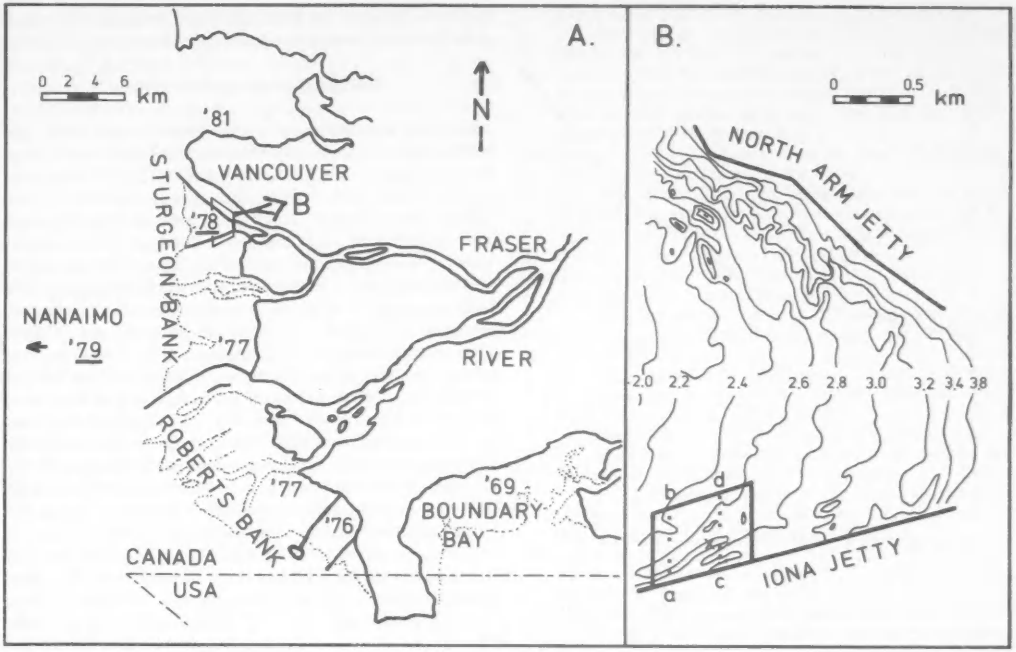

FIG. 3. A. Year of first recorded collections of *Zostera japonica* in southwestern British Columbia. Dates are underlined at sites where observations were made the previous year and no plants were found. Area within the parallelogram on northern Sturgeon Bank is enlarged in B. B. Intertidal flats on northern Sturgeon Bank. Contours of bottom elevation are in metres above chart datum. Box north of Iona Jetty indicates study site. Dots on lines a–b and c–d are sites at which establishment of *Z. japonica* was monitored; "x" is the site where growth was measured. (A is modified after Tomlins and Thomson (1980); B is modified after Harrison (1981).)

TABLE 1. Details of Iona Island sites at which the establishment of *Zostera japonica* was monitored in summer 1980.

Site	Distance (m) north of Iona jetty	Elevation (m) above chart datum	Sediment type	Description	Occurrence of plants
a–b 1	10	2.6	Mud	Tide pool	+
2	35	2.9	Sand	Wave crest	–
3	70	2.4	Mud	Wave trough	+
4	100	2.6	Sand	Wave crest	–
c–d 1	5	2.8	Mud	Tide pool	–
2	35	2.7	Mud	Wave trough	+
3	70	2.7	Mud	Tide pool	+
4	115	2.7	Mud	Between trough and crest	+
5	215	2.5	Sandy mud	Wave trough	+

vegetative and flowering plants are uprooted in autumn and form loose mats in the intertidal zone. Occasionally floating shoots can be seen on the surface of deeper water, but it is not known whether such plants can become established if they are subsequently deposited on shore. Man may inadvertently transport these plants when they become entangled on boating or fishing gear. Other vectors may be birds which graze on seed-bearing plants and defecate undigested seeds later, often far from the feeding site. Ridley (1930) suggested that *Z. marina* seeds can pass through mallards (*Anas platyrhynchos*) and retain their viability. Locally, the brant (*Branta nigricans*) is commonly observed feeding in intertidal seagrass beds but its role as a vector has not been determined. In the Netherlands, however, consumption of intertidal *Z. noltii*

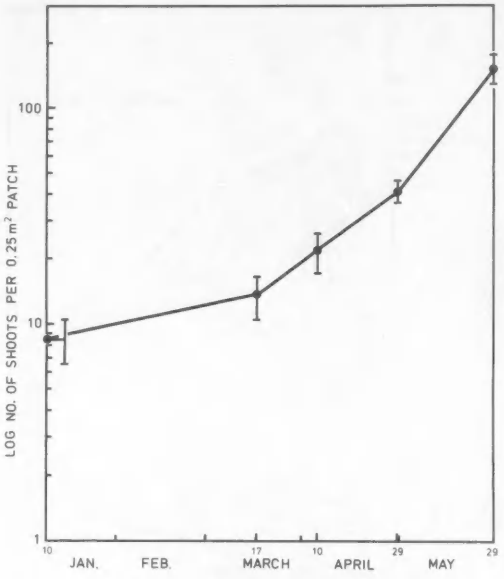

FIG. 4. Mean numbers of shoots (±SD) in 0.25-m^2 patches of *Zostera japonica* at four intertidal sites on northern Sturgeon Bank during winter and early spring 1979.

by *Branta bernicla* and three species of *Anas* was estimated as 26% of the annual plant production (Jacobs et al. 1981).

Establishment and Growth

Invading populations were monitored in both 1979 and 1980 on the mixed sand and mud, intertidal flats west of Iona Island (located at 49°12.8′N; 123°13.5′W, Fig. 3B). Near the Iona jetty, the gently sloping tidal flats are marked by sand waves with mud-filling depressions between the crests and with isolated pools remaining at low tide (Fig. 3B). In winter 1979, four permanent quadrats (0.25 m^2) containing small overwintering shoot populations were established on mud at an elevation of 2.6 m above chart datum, and the numbers of shoots were recorded from early January to the end of May. In spring and summer 1980 the success of individual patches (at least one shoot) was determined from periodic surveys of circular plots (10-m diameter) on both sand and mud at a variety of elevations (Table 1, Fig. 3B). Even when plants were absent initially at a site, observations were continued to determine if plants could invade these sites later during the summer. A quadrat divided into 5 × 5 cm squares was placed over the sediment and the number of squares occupied by plants was used as an estimate of the area of the patch.

From a mean of 8.5 overwintering shoots/0.25 m^2, the patches grew to over 150 shoots in 4.5 mo (Fig. 4). Few new shoots appeared until late March, but after that time growth was exponential until late May when the high density made counting in situ inaccurate, and the patches were harvested. Similar rapid rates of expansion of patches were reported by Arasaki (1950b). At the time of harvest, the mean (±SD)

TABLE 2. Areas (cm^2) and gains and losses of patches of *Zostera japonica* in 10-m circles around sites on the Iona Island intertidal sediments during summer 1980.

					Lost patches	
Site[a]	Month[b]	No. of patches	Area ($\bar{x}$ ± SD)	No. of new patches	No.	Mean area at last sampling
a–b 1	M	5	382±222.5	—[c]	—	—
	J	5	670±435.0	0	0	—
	A	5	1280±677.4	0	0	—
a–b 3	M	4	520±504.2	—	—	—
	J	5	470±467.2	1	0	—
	A	5	997±1110.0	0	0	—
c–d 2	M	30	113±170.4	—	—	—
	J	21	204±259.6	2	11	34±12.6
	A	18	228±257.3	3	6	86±94.6
c–d 3	M	6	126±190.6	—	—	—
	J	5	242±384.4	2	3	25±0.0
	A	3	1058±866.1	0	2	25±0.0
c–d 4	M	22	106±114.8	—	—	—
	J	22	117±137.6	5	5	50±55.9
	A	21	192±184.6	5	6	58±70.1
c–d 5	M	6	25±0.0	—	—	—
	J	6	107±78.1	0	0	—
	A	12	162±154.6	7	1	25

[a]For locations, see Table 1 and Fig. 3B.
[b]M = May, J = June, A = August.
[c]Not applicable.

biomass of shoots was 4.4 (±1.46) g dry wt/0.25 m^2 in the patches of which flowering shoots accounted for up to 70%. Biomass of rhizomes and roots was only 1.0 (±0.38) g, an average of 17% of the total biomass. No attempt was made to separate quantitatively the seedlings from mature (overwintering) shoots although qualitative observations of the rhizomes indicated that the shoots were derived both from seedlings and from branching of existing plants.

No plants were found in the higher plots (above 2.5 m above chart datum on sand and above 2.7 m on mud) in 1980 and the existing populations were variable in space and time (Table 2). Many small patches which were present in May were gone by June; only 4.3% of the seedlings which had not grown beyond the one to two shoot stage by May were successfully established. Those patches which survived until June had increased an average of 100% in area in the intervening month and they grew 167% more in the next two months. Few losses occurred between June and August, but some new patches appeared in both summer samples. All new plants found in the surveys were seedlings. Arasaki (1950b) reported germination only between February and April in Japan, but Harrison (1982c) found some seedlings in June and July on Roberts Bank, B.C. Together these observations suggest that the chance of an individual seedling becoming established is small and is partly dependent on the elevation of the bottom and the nature of the sediment, but that once securely rooted the plant can quickly expand by extension and branching of the rhizome.

Discussion

The introduction and spread of *Z. japonica* on intertidal sediments in southern British Columbia and adjoining USA represents an expansion of the already valuable seagrass habitat provided by *Z. marina*. Although both in Japan and in North America *Z. japonica* can grow in shallow water intermixed with *Z. marina* (Arasaki 1950a; Harrison 1982a), Harrison (1982b) concluded from culture experiments that *Z. japonica* is not likely to displace existing *Z. marina* beds nor can *Z. marina* compete successfully in the intertidal zone favored by *Z. japonica*. The two seagrasses differ not only in their preferred habitats but also in their life histories: the invader is an annual or short-lived perennial with a high seed production whereas the native eelgrass is a longer-lived perennial with a greater emphasis on vegetative reproduction. At present, *Z. japonica* occupies only a small fraction of its potential habitat in North America and we predict significant changes in the ecology of intertidal sediments as the seagrass spreads.

Acknowledgments

Financial support for this project came from the Canadian National Sportsmen's Fund, the North Cascades Audubon Society, and the Natural Sciences and Engineering Research Council of Canada. Able field assistance was provided by R. Haigh, S. Hollenberg, and A. Listrum. Dr R. C. Phillips kindly drew our attention to collections of *Z. japonica* in Oregon.

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Comparison of Searching Behavior for Zooplankton in an Obligate Planktivore, Blueback Herring (*Alosa aestivalis*) and a Facultative Planktivore, Bluegill (*Lepomis machrochirus*)

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JANSSEN, J. 1982. Comparison of searching behavior for zooplankton in an obligate planktivore, blueback herring (*Alosa aestivalis*) and a facultative planktivore, bluegill (*Lepomis machrochirus*). Can. J. Fish. Aquat. Sci. 39: 1649 – 1654.

Studies of zooplankton feeding by juvenile blueback herring (*Alosa aestivalis*), an obligate planktivore, and juvenile bluegills (*Lepomis macrochirus*), a facultative planktivore, indicate that they search by markedly different methods. The herring searches as it swims, sighting most prey above its horizontal course, and swims up to take the prey. The bluegill searches while stopped (hovering). If a prey is sighted, it swims to it, sucks it in, then coasts to a stop to resume searching. If no prey is sighted, it swims a short distance, coasts to a stop, and searches again. For uniform size prey (*Artemia salina* first instar nauplii) the time the bluegill hovers in a particular search position is positively correlated with the distance the prey is from the fish (r^2 = 0.69–0.77, four fish). This, in conjunction with previous work on bluegills, suggests they take the first prey item seen, rather than choosing between prey. The bluegill is more reactive to moving prey of *Daphnia* size; however, blueback herring do not distinguish between motile and nonmotile prey, perhaps because any prey's image is moving across its retina as the herring swims. Stopping to search may be adaptive where there is a heterogenous (weedy) background, whereas moving prey are detected against the stationary background. Such a background is likely confusing to a moving fish.

Key words: blueback herring, *Alosa aestivalis*; bluegill, *Lepomis macrochirus*; predation, zooplankton

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Des études sur l'alimentation zooplanctonique de jeunes aloses d'été (*Alosa aestivalis*), un planctophage obligatoire, et de jeunes crapets arlequins (*Lepomis macrochirus*), un planctophage facultatif, indiquent que ces deux espèces ont des méthodes notablement différentes de recherche de leur nourriture. L'alose d'été recherche la sienne en nageant, apercevant la plupart de ses proies au-dessus de sa course horizontale, et nage vers le haut pour les saisir. Le crapet arlequin, pour sa part, recherche sa proie en nage stationnaire. Quand il en aperçoit une, il se dirige vers elle, l'aspire et nage sur sa lancée, pour s'arrêter et reprendre son guet. S'il n'y a pas de proie en vue, le poisson nage sur une courte distance, continue en nage libre, s'arrête et continue à chercher. Dans le cas d'une proie de taille uniforme (premiers stades nauplius d'*Artemia salina*), le temps consacré par un crapet arlequin à une nage sédentaire en un endroit particulier est en corrélation positive avec la distance séparant la proie du poisson (r^2 = 0,69 à 0,77, quatre poissons). Cette observation, concurremment avec des travaux antérieurs sur le crapet arlequin, donne à penser que ce poisson ingère la première proie aperçue, sans discrimination. Il réagit davantage devant une proie mobile de la taille de *Daphnia*. Par contre, l'alose d'été ne distingue pas entre proies mobiles et immobiles, peut-être parce que l'image de la proie traverse la rétine d'un poisson en mouvement. Le fait de s'arrêter pour rechercher sa proie peut avoir une valeur adaptive dans un milieu hétérogène (herbeux) et quand cette proie est détectée contre un fond stationnaire. Un tel fond est de nature à dérouter un poisson en mouvement.

Received March 23, 1982
Accepted August 15, 1982

Reçu le 23 mars 1982
Accepté le 15 août 1982

BROOKS and Dodson's (1965) study of the effects of two obligate zooplanktivores, the alewife (*Alosa pseudoharengus*)

Printed in Canada (J6883)
Imprimé au Canada (J6883)

and blueback herring (*A. aestivalis*) on zooplankton in New England lakes focused considerable attention on predation and zooplankton community structures (see Kerfoot 1980; Zaret 1980). Most of the work on fish feeding on zooplankton has used readily available facultative planktivores such as sunfishes (*Lepomis* spp.) (Werner and Hall 1974; Confer and Blades 1975; O'Brien et al. 1976) rather than herrings or other obligate planktivores presumably because the obligate planktivores are extremely delicate and difficult to capture alive. It would not be surprising to find that an obligate planktivore is, by some measure, such as feeding rate, more "competent" than a facultative planktivore. What is surprising is that *Alosa*'s search movements are very different from that of bluegill (*Lepomis macrochirus*). The *Alosa* species search while swimming, whereas the bluegill searches only when hovering fixed relative to the landscape.

The premise of this research is that the adaptiveness of the two search modes is situation specific. Bluegills, particularly the juveniles, are mostly restricted to littoral areas where the background is visually heterogenous because of weeds, etc. In such a situation, it should be an advantage for the fish to hold its head stationary relative to the landscape to detect movement better. Blueback herring live only in pelagic areas where the background is fairly homogenous and stationary search is not necessary. I have given a description of search in the two species with more detailed description of the use of space and time by the bluegill. I have included experiments on the effect of prey motion and weeds on the two species. The results suggest that bluegills take what they see first, use prey motion, and can find prey among weeds. Blueback herring appear not to use prey motion to locate prey and are strongly inhibited by even small pieces of weeds.

Materials and Methods

Blueback herring were seined pelagically from Par Pond, Savannah River Plant, Aiken, SC, in September, 1980. Four fish were used for most experiments. They were about 75 mm tail length (TL) at the time of the experiments, and were kept in a glass aquarium 89 cm (L) × 45 cm (W) × 39 cm (D) with slate bottom. The outside of the aquarium was covered on three sides with white paper and one long side was open for viewing and videotaping (Sanyo VTR) and a blind built around it.

Bluegills (30−80 mm) were from the Orangeburg National Fish Hatchery, Orangeburg, SC, collected in September, 1980. These were kept in a variety of aquaria.

Four bluegills (43−50 mm), used for experiments on use of time and space, were kept in a 72 cm (L) × 30 cm (W) × 20 cm (D) glass aquarium with white sand covering the slate bottom. Three sides were covered with white paper and one long side was uncovered for viewing and videotaping. Within the aquarium, fish were confined to an area 72 cm L × 12 cm W with Plexiglas, to keep most movement parallel to the long axis of the aquarium. Only one bluegill was used at a time, each was kept alone in the aquarium for 7−14 d prior to the experiment and fed on *Artemia* nauplii and *Daphnia*. For the experiment I introduced 300 *Artemia* nauplii (size range: 0.69−0.74 mm) to the confined space (initial density about 174/L). The video system recorded only the center 25 cm of the aquarium to eliminate any end wall effect on fish movement. I recorded for 3 min during which time no one was in the aquarium room. The videotape was replayed by $\frac{1}{4}$ speed for analysis. Measurements of position and time were made only when the fish moved along the long axis of the aquarium. The background for all prey taken for such movements was white regardless of whether the prey was horizontally located or above or below the fish.

I tested the importance of prey motion to blueback herring by gluing with Permabond® superglue, live *Daphnia magna* of various sizes and lake chubsucker (*Erimyzon sucetta*) eggs to strips of transparent acetate (0.1 mm thick). For an experiment, one prey was glued to each of two strips. Prey were measured under the microscope and pairs were indistinguishable in size to me. One prey was fixed motionless in the aquarium, the other manipulated by hand in an up−down pattern similar to a *Daphnia* sp. of similar size. I added several hundred *Daphnia* to the aquarium, then introduced the glued prey. For egg experiments, 10−20 eggs were added in pulses; these slowly sank. The free-swimming *Daphnia* and loose eggs provided an alternate prey source from the glued prey so blueback herring did not attempt to feed on glued prey because they were the only prey available. The free-swimming *Daphnia* and scattered eggs also kept the fish moving around the aquarium. The fish fed on the free prey, while swimming back and forth the length of the aquarium. On most such sweeps, one or more of the fish attempted to eat a glued prey. I recorded which prey was attacked; only the first attacked in a sweep was recorded, in case fish interfered with each other. I determined which glued prey was to be moved by consulting a table of random numbers (odd vs. even) before the fish approached on a sweep.

I used *Daphnia* glued to acetate sheets to test the effect of prey motion on bluegills also. The design for bluegills and blueback herring are not strictly comparable as bluegills are solitary hunters (and were kept solitary) and tend to concentrate search in a particular area rather than swimming rapidly around the tank. As a result they make rapid repeated attempts to remove glued prey, give up, and leave the area. I therefore presented several hundred free-swimming *Daphnia* to a bluegill and when it began to feed I introduced two glued prey at the end of the aquarium opposite the fish. One of the prey was moved, determined as in the experiment with blueback herring, and I recorded which of the glued prey was attacked by the bluegill. The experiment was repeated seven times (over several days) for each of seven bluegills (37−62 mm).

Experiments on the effect of vegetation were performed primarily on blueback herring as bluegills did not respond to nonmotile glued prey. In the most important experiment I glued five lake chubsucker eggs or equal-sized *Daphnia* to an acetate sheet. The end prey each had four aerial leaves of *Myriophyllum brasiliense* glued around it, two above and two below. Several hundred free-swimming *Daphnia* or 10−20 loose eggs in pulses were added to stimulate feeding, and the acetate array was introduced at one end. The experiment was repeated with the leaves around one of the glued prey removed and repeated again with all leaves removed to check for position effects. Shorter experiments and corroborative observations are described with the results.

TABLE 1. Use of time and space by bluegills. See text for definition of search−quit and search−pursue times and search−search and pursuit distances. TL = total length, r^2 = product−moment coefficient of determination of search−pursue times and pursuit distances. Values given outside parentheses are the mean, those within parentheses represent the range.

		Times (s)		Distances (mm)		
Fish	TL	Search−quit	Search−pursue	Search−search	Pursuit	r^2
1	47	1.5(0.9−3.0) $n = 18$	0.5(0.1−1.7) $n = 25$	27(12−57) $n = 18$	34(14−112) $n = 25$	0.69 $n = 25$
2	50	1.2(0.7−2.3) $n = 10$	0.3(0.1−0.7) $n = 34$	35(30−57) $n = 10$	19(4−49) $n = 34$	0.77 $n = 34$
3	46	1.2(0.8−2.6) $n = 19$	0.4(0.1−1.1) $n = 30$	28(16−59) $n = 19$	21(4−83) $n = 30$	0.70 $n = 30$
4	43	0.9(0.7−1.7) $n = 14$	0.6(0.1−1.1) $n = 29$	28(17−50) $n = 14$	26(8−66) $n = 29$	0.76 $n = 29$

Results

DESCRIPTION OF SEARCH BEHAVIORS

Swim-search (SS) — Search by the blueback herring for individual prey is similar to that of the alewife (*A. Pseudoharengus*; see Janssen 1976a). A prey item is detected as the fish swims, usually above the horizontal swimming track, and after the prey is taken the fish resumes its horizontal track (Janssen 1981). Eye movements are usually not apparent; however, when approaching elusive prey such as calanoid copepods, the eyes are rotated forward until the prey is taken, presumably to increase binocularity. Cruising speed as the fish feeds is difficult to determine as it is punctuated with feeding movements. My estimates (from videotape) are about 8−10 cm/s for 75-mm fish. When not feeding, the speed is about 3−4 cm/s and the fish makes several tail beats, then coasts.

Hover-search (HS) — The bluegill hovers in stationary positions as it searches for zooplankton. It uses delicate movements of its pectoral, soft dorsal, caudal, and soft anal fins as it hovers. Head motion is not noticeable. If no prey is detected it swims a short distance (straight course, usually horizontally), coasts to a stop (no braking), then searches. If a prey is detected, it turns and swims straight to it and engulfs it as it coasts to a stop. It then immediately begins to search. If the prey is taken above or below the fish's search position, the fish reorients to a horizontal position before searching. If the prey actively swims, the fish will follow it once it is detected, but I have never seen a bluegill alter its swimming track because it detected a prey while swimming. Eye movements are usually not discernible. However, the eyes are rotated forward when following elusive prey, and for prey along the bottom, apparently to increase binocularity. When the fish turns, the eyes are rotated in the direction of the turn until complete.

USE OF SPACE AND TIME BY THE BLUEGILL

The search of the bluegill can be partitioned as follows:

Pursuit distance. The distance the fish swims to the prey from the search position.

Search−search distance. The distance between consecutive search positions when no prey is taken.

Search−quit time. The time the fish stays at a search position before moving to another when no prey is sighted.

Search−pursue time. The time the fish stays at the search position before it begins to pursue prey.

The absolute values (Table 1) of these measurements of distance and time are not important as they will probably change with fish and prey size, type, water clarity, illumination, and other factors. However, comparisons and correlations are important. Search−quit times are significantly longer than search−pursuit times (Table 1, $P < 0.001$, Mann Whitney U-test). This suggests that bluegills move to take a prey when it is first detected and do not wait to inventory all prey that could possibly be detected. If closer prey are seen before more distant prey and if the fish takes what it detects first, pursuit distance and search−pursue time should be positively correlated. The amount of variance in search time explained by pursuit distance (r^2, Table 1) is highly significant for all fish tested ($P < 0.001$).

EFFECT OF PREY MOTION

Prey motion had no effect on food choice by blueback herring (Table 2). In no experiment was there a statistically significant preference for moved or nonmoved prey ($P > 0.25$, chi-square test). The results suggest that blueback herring are not sensitive to prey movements of the size and type *Daphnia* make, perhaps because the fish is moving relative to the prey so there is always movement of the prey's image across its retina. Even for stationary prey the image is likely to oscillate as the fish's head oscillates to swim. The ability to locate nonmotile prey must be important to blueback herring as most of their prey in native marine habitats are calanoid copepods which move only occasionally (Brooks 1968).

For bluegills, in each case, the fish repeatedly attacked the moved *Daphnia* and ignored (or did not notice) the one not moved. Eventually each of the seven fish ceased attacking the

TABLE 2. Effect of prey motion on prey selection by blueback herring. Each experiment used two equal-sized prey, *Daphnia magna* or lake chubsucker eggs, each of which was glued (Permabond® superglue) to a separate clear acetate sheet. One of the two prey was held stationary while the other was moved. See text for further details.

Experiment	Prey size, mm	Prey selected	
		Moving	Stationary
		Daphnia	
1	2.8	12	12
2	2.0	23	19
3	1.0	5	6
4	1.0	20	17
		Lake Chubsucker eggs	
5	1.6	18	23

moved prey and resumed feeding on the free-swimming *Daphnia*. The results indicate selection for motile prey ($P = 1/64$ for each fish, two-tailed binomial test). If presented with only nonmoved prey the bluegills continued to feed on the free-swimming *Daphnia*, but never attacked those glued.

EFFECT OF VEGETATION

Leaves of *Myriophyllum* greatly interfered with blueback herring feeding (Table 3). In experiments 1, 2, 4, and 5, selection was always highest for that *Daphnia* or egg furthest from the leaves ($P < 0.001$), but in experiment 3 where no leaves were present there was no selection for any site ($P > 0.5$, chi-square test). Thus, even very sparse vegetation inhibits feeding.

When sprigs of *Myriophyllum* were spread loosely over half the aquarium surface the blueback herring took 47 of 50 *Daphnia* from the half without weeds. The other three were from near the bottom of the weeded side.

Although these experiments demonstrate that blueback herring avoid weeds when feeding, I am unable to provide exactly comparable data for bluegills because they react poorly to nonmotile prey. However, bluegills would venture into weeds in pursuit of *Daphnia* and they have taken *Daphnia* that had one antenna glued to a *Myriophyllum* leaf. Such *Daphnia* maintain a jerky but very sporadic movement. The bluegill attacks the *Daphnia* only after a "jerk." It is possible that the heterogenous background provided by weeds inhibits search by bluegills too. Nevertheless, juvenile bluegills actively seek weedy cover and appear to be specialists in foraging there (Werner and Hall 1976, 1977, 1979).

Discussion

Herrings are well known as obligate planktivores, very abundant pelagically in coastal areas. We might expect herring to use a very effective search method for zooplankton. Why then do bluegills search in the strikingly different hover–search technique? I suggest that hover–search used by bluegills is adapted to heterogenous backgrounds where detection of prey motion is important and swim–search as used by herring is useful where the background is not heterogenous.

Facultative planktivores such as bluegills are planktivorous in their juvenile stage. Much of their foraging is in the littoral zone where the background is often broken by vegetation and seston is more prevalent. Vegetation is also used as cover. If bluegills have "motion detectors" as part of their visual processing systems to help them locate prey, these would likely be falsely stimulated if the fish searched while swimming as images of background objects would move across the retina. Motion detectors have been found in a variety of fishes (Jacobson and Gaze 1964; Cronly-Dillon 1964; Maksimova et al. 1971) as well as in anurans (Lettvin et al. 1959; Muntz 1977). Anurans are stationary when searching and do not move their eyes (Muntz 1977). Pigarev et al. (1971) found that the prey detection system of frogs is inactive when they jump but other visual processing systems are active. When searching, bluegills did not move their eyes (if at all) until prey was seen. Any eye (or body) movement probably inhibits detection of movement (Muntz 1977). I watched several hundred juvenile bluegills feeding on mixed zooplankton introduced into an artificial stream with 7 cm/s flow. Search was by HS but when the fish coasted to a stop, they would drift back about 1 cm, then hold position. This suggests that they initially stop relative to the water, but ultimately orient relative to the landscape. I have also watched juvenile bluegills searching while moving mostly parallel to wave fronts impinging on a shoreline. As they hovered, their heads were motionless relative to the bottom, a feat which required an unusual amount of body oscillation to compensate.

Blueback herring and other SS fish are also likely to have motion detectors. A motion detector detects movement of an image across the retina. As a blueback herring swims, images of prey and non-prey will move across the retina, unless (and until) the prey is directly in front of the fish. As blueback herring search above their swimming track (Janssen 1981), their prey are never directly in front of them until they swim up to take it. Then the image grows as the fish approaches. Because the fish moves, the motion of prey may be unimportant to blueback herring detection of prey. It also means that they must rely on other cues to distinguish prey from seston. Interestingly, Volkmann (1974) had difficulty training goldfish (*Carassius auratus*) to distinguish moving and nonmoving objects. Goldfish use SS (personal observation), so from their perspective, both objects may have been "moving."

Some experiments with other fish are supportive. Meesters (1940, cited by Hyatt 1979) found that northern pike (*Esox lucius*), a hovering ambush piscivore, attack only moving prey. Larval *Coregonus wartmani* (presumably SS as in its congeners, personal observation) do not require prey movement (Braum 1967). However, Hyatt (1979) found prey motion was important to sockeye salmon (*Oncorhynchus nerka*) [presumably SS, but prey were large (6–10 mm)]. SS fish certainly can detect movement of larger objects such as conspecifics and predators. Also, even though my experiment indicates movement similar to a *Daphnia* hop and sink is not necessary to locate prey, it does not necessarily demonstrate that they are insensitive to such movement.

Search in birds provides a useful comparison. Swifts (Apodidae) and swallows (Hirundididae) search for aerial

TABLE 3. Effect of vegetation on prey selection by blueback herring. Five prey (*Daphnia magna* or lake chubsucker eggs) were glued (Permabond® superglue) to an acetate sheet. In experiments 1 and 4 the two end prey were surrounded by four aerial leaves of *Myriophyllum brasiliense* (denoted by asterisk), glued to the acetate. One set of leaves was removed in experiments 2 and 5; both sets were removed in experiment 3. See text for further details.

Prey position and distance from position C (cm)	Number of capture attempts				
	A(5)	B(2)	C(0)	D(2)	E(5)
Exp. No.	*Daphnia (2.7–2.8 mm)*				
1	0*	5	24	8	0*
2	0*	2	4	7	10
3	6	4	6	7	5
	Eggs (1.6 mm)				
4	0*	7	22	10	0*
5	0*	0	5	19	Prey removed by fish

insects while flying, and seek open areas. Flycatchers (Tyrannidae) frequently forage in forests and are perched as they search. American robins (*Turdus migratorius*) and European blackbirds (*Turdus merula*, Smith, 1974) search while stopped, head motionless, then run to a new location if no prey is seen. This search is very similar to the bluegill. Some ground birds do search while walking, but these use "head bobbing." In the rock dove (*Columba livia*) the head is stationary relative to surrounding objects as it moves backwards relative to the walking body (Frost 1978).

Several models of foraging by planktivores have been proposed (Werner and Hall 1974; O'Brien et al. 1976; Confer and Blades 1975; Eggers 1977). Such models require information such as swimming speed and reactive distance to various prey. Some of the models incorporate "choice" of prey. It should be clear from the present results that one model will not be adequate for all fishes. For example, a blueback herring is probably searching all the time that it is not pursuing a sighted prey. However, the total search time of bluegills is partitioned into time actually searching and time moving between search locations.

While we know that fish have motion detectors, we know little about how they relate to foraging. More is known about how the prey-detection system in anurans relates to feeding. Pigarev and Zenkin (1970) showed that a frog presented with one stimulus is not distracted by a second stimulus; i.e. they attempt to take the first prey detected. Using information about frog neuroanatomy and neurophysiology, Didday (1976) proposed a specific mechanism in which neurological activity evoked by one prey effectively "shuts off" the rest of the prey detection system. This probably explains the observation of Freed (1980) that tree frogs (*Hyla cinerea*) took much smaller, more active prey as opposed to large, relatively nonmotile prey. The reason is probably because the motile prey more frequently activated the frog's motion detectors.

The proposal that the bluegill takes what it first detects is consistent with previous work on bluegills feeding on *Daphnia* in which it is assumed that the fish inventories the prey, than makes a decision. The "first sight" hypothesis is behaviorally simpler and has precedence with the work on anurans. Werner and Hall (1974) proposed that bluegills choose the most energetically rewarding prey to optimize energy intake. O'Brien et al. (1976) proposed the simpler model that bluegills choose the prey with the largest "apparent size." Both models were reasonably successful, but present results suggest that bluegills attempt to capture what they detect first, thus make no choice as other prey are not detected. Apparent size is the angle a prey covers on the fish's retina and is a function of prey distance and prey size (O'Brien et al. 1976). However, the prey with the largest apparent size is most likely to be seen first, hence the experimental data of O'Brien et al. are consistent with my simpler hypothesis. Zaret and Kerfoot (1975) suggested that it is visibility that determines what prey are taken, not size of prey. Kerfoot (1982) showed that pumpkinseeds (*Lepomis gibbosus*) consistently "chose" 1.8-mm *Daphnia* over 8-mm *Chaoborus* larvae even though both prey were close enough to be seen. *Chaoborus*, while larger, is highly transparent with the result that the much smaller *Daphnia* is more easily seen. This observation is clearly consistent with my hypothesis that bluegills take what they detect first. Note that my proposal is not the same as an encounter model; the bluegill will bypass potentially encountered prey as it swims to the first sighted prey. The "first sight" hypothesis is also consistent with the observation that prey motion enhances prey visibility to bluegills (Confer and Blades 1975; O'Brien 1979).

We should not expect all planktivores to affect zooplankton communities similarly. A variety of capture techniques exist (see Janssen 1980; Drenner and McComas 1980) and, as shown here, search techniques are also variable. Drenner et al. (1978) have found that lakes with an abundance of gizzard shad (*Dorosoma cepedianum*) tend to be dominated by evasive zooplankton. Calanoids may be able to coexist with bluegills. In enclosures, Lynch (1979) found *Diaptomus siciloides* survived bluegill predation. Washington Park Pond in Milwaukee, Wisconsin, contains many stunted bluegills and a near monoculture of *Diaptomus* during the summer; plankton tows of thousands of *Diaptomus* have only several other microcrustacea (personal observation). The survival of *Diaptomus* is probably due to low visibility from a lack of constant motion (Brooks 1968) and high escape capacity (Janssen 1976b; Drenner et al. 1978). Par Pond is in direct

contrast to this; calanoids are absent except during the winter (personal observation). Elimination of calanoids from lakes by alewives and blueback herring has been reported elsewhere (Brooks and Dodson 1965; Hutchinson 1971). This is perhaps because alewives and blueback herring have an adequate search mechanism for relatively immobile planktonic prey. Zooplankton communities are interestingly complex in their organization and dynamics; fish contribute to this complexity and are themselves diverse in how they interact with zooplankton.

Acknowledgments

I thank J. P. Giesy, W. J. O'Brien, J. Breck, L. A. Martínez, and M. Peror for reviews. This work was supported by Contract DE-AC09-76SRO0819 between the U.S. Department of Energy and the University of Georgia.

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Distribution and Activity of Attached and Free-Living Suspended Bacteria in the Bay of Fundy[1]

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CAMMEN, L. M., AND J. A. WALKER. 1982. Distribution and activity of attached and free-living suspended bacteria in the Bay of Fundy. Can. J. Fish. Aquat. Sci. 39: 1655 – 1663.

We investigated the distribution of suspended bacteria in the Bay of Fundy on cruises in the spring, summer, and fall of 1979; a total of 22 stations were sampled throughout the Bay at the surface, midwater, and bottom. Free-living bacteria dominated the lower Bay in all seasons ($0.2–4.0 \times 10^6$ cells $\cdot$ mL^{-1} compared to $0.0–0.5 \times 10^6$ attached cells $\cdot$ mL^{-1}). Attached bacteria were more important in the upper reaches of the Bay with numbers as high as 2×10^6 cells $\cdot$ mL^{-1} accounting for up to 94% of the total bacteria. Both the abundance and proportion of attached bacteria were strongly correlated with the concentration of suspended particulate matter. Average cell volume was not significantly different between spring (0.11 μm^3, surface water only) and summer (0.09 μm^3, all depths) and was similar for free-living and attached cells.

Bacterial activity in summer and fall, based on uptake of [^{3}H]glutamic acid, generally followed the same pattern as cell numbers. Mean turnover time for glutamic acid in surface water was 64 h in summer and 205 h in fall. On a per cell basis, free-living bacteria in surface water were about twice as active as attached cells during summer, but only half as active during fall. The proportion of total activity due to attached cells increased with increasing substrate concentration.

Key words: suspended bacteria, attached bacteria, free-living bacteria, heterotrophic activity, suspended particulate matter

CAMMEN, L. M., AND J. A. WALKER. 1982. Distribution and activity of attached and free-living suspended bacteria in the Bay of Fundy. Can. J. Fish. Aquat. Sci. 39: 1655 – 1663.

Lors de croisières de printemps, d'été et d'automne 1979 dans la baie de Fundy, nous avons étudié la répartition des bactéries en suspension; un total de 22 stations à la grandeur de la baie ont été échantillonnées, en surface, entre deux eaux et au fond. Les bactéries libres prédominent en toutes saisons à l'entrée de la baie ($0{,}2–4{,}0 \times 10^6$ cellules $\cdot$ mL^{-1}, comparativement à $0{,}0–0{,}5 \times 10^6$ cellules fixées $\cdot$ mL^{-1}). À la tête de la baie, les bactéries fixées sont plus abondantes, des nombres aussi élevés que 2×10^6 cellules $\cdot$ mL^{-1} constituant jusqu'à 94 % des bactéries totales. Il y a étroite corrélation entre l'abondance et la proportion des bactéries fixées, d'une part, et la concentration de matière particulaire en suspension, d'autre part. Le volume moyen de cellules ne diffère pas significativement au printemps (0,11 μm^3, eaux de surface seulement) et en été (0,09 μm^3, toutes profondeurs) et est le même pour les cellules libres que pour les cellules fixées.

L'activité bactérienne en été et en automne, telle qu'indiquée par la captation d'acide [^{3}H]glutamique, se comporte en général de la même manière que le nombre de cellules. Le temps de renouvellement moyen de l'acide glutamique dans l'eau de surface est de 64 h en été et de 205 h en automne. Sur la base de cellules individuelles, les bactéries libres dans l'eau de surface en été sont à peu près deux fois plus actives que les bactéries fixées, mais deux fois moins actives que ces dernières en automne. La proportion d'activité totale des cellules fixées augmente à mesure qu'augmente la concentration de substrat.

Received March 3, 1982
Accepted August 30, 1982

Reçu le 3 mars 1982
Accepté le 30 août 1982

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Printed in Canada (J6830)
Imprimé au Canada (J6830)

ALTHOUGH the Bay of Fundy is well known for its unique hydrography, very little has been known about the biology and chemistry of the water column. However, with recent consideration of possible tidal power projects in the Bay, research interest in all areas has increased and in 1978–80 a series of seasonal cruises through the Bay was undertaken to provide some basic biological and chemical information. We present here data from three of these cruises on the distribution and activity of suspended bacteria in the Bay.

Suspended bacteria may exist as either free-living cells or attached to particles. The proportion of cells that are attached may vary from virtually none to most of the suspended bacteria depending on location (reviewed by Hanson and Wiebe 1977; Kirchman and Mitchell 1982; Linley and Field 1982). We investigated the extent of attachment in the Bay and whether it varied seasonally and with the concentration of suspended particulate matter (SPM).

The effect of attachment of bacterial cells on activity remains unclear partly because some investigators have used ATP as a measure of microbial abundance with which to standardize activity measurements, whereas others have used some form of direct cell counts of bacteria. Typically, total uptake of a labeled substrate is separated into that due to organisms retained on 3-μm filters and that due to organisms < 3 μm but retained on 0.2-μm filters. However, when microbial abundance is measured with ATP, the >3-μm fraction includes not only bacteria attached to particles, but also phytoplankton, large protozoans, and even zooplankton which may not be able to use the substrate at natural levels. Thus, the fact that activity per unit ATP may be greater in the <3-μm fraction than in the >3-μm fraction (Azam and Hodson 1977; Hodson et al. 1981a) does not necessarily give any useful information about the relative activity of free-living and attached bacteria (as these authors were aware, their research did not deal with bacteria per se). Studies of attached cells were either less active (Bell and Albright 1981, bacteria by direct counting have shown that on a per cell basis attached cells were either less active (Bell and Albright 1981, 1982), as active (Schleyer 1981b), or more active (Hodson et al. 1981b; Kirchman and Mitchell 1982; Paerl and Merkel 1982) than free-living cells.

Wangersky (1977) has argued that bacteria attached to particles should be more active than free-living bacteria because the particles should move somewhat independently of the water around them, thus continually renewing the supply of nutrients to the cells on their surface, whereas free-living bacteria would be largely constrained to the nutrients in the small envelope of water accompanying them. In addition, particles may serve to concentrate dissolved organic matter and micronutrients from the surrounding water and thus provide an enriched microenvironment for the attached bacteria (Jannasch and Pritchard 1972; Paerl and Merkel 1982). During the summer and fall cruises, we also attempted to evaluate the effect of attachment to particles on bacterial activity.

Methods and Materials

Sampling and Analytical Procedures

The data presented here were collected on three cruises (79-004, March 28–April 6; 79-023, August 11–18; and 70-034, October 28–November 10, 1979) of the CSS *Dawson* in the Bay of Fundy. Nineteen stations were occupied during each cruise (Fig. 1). Throughout this paper we refer to both the lower Bay of Fundy, which includes stations 1–16, and the upper reaches of the Bay, which includes stations 17–22. At each station, samples of surface, midwater (exactly midway between the surface and bottom samples), and bottom water were taken with 5-L Niskin bottles; water depth ranged from about 200 m near the mouth of the Bay to as little as 17 m in the upper reaches. After inverting the bottles several times, we subsampled for bacteria, suspended particulate matter (SPM), particulate organic carbon (POC), and chlorophyll *a*; in addition, at some stations during the summer and fall cruises, incubations were carried out to measure heterotrophic activity.

SPM was determined by filtering 1 L or less of seawater through tared, 0.45-μm Nuclepore filters. POC was collected on precombusted, 0.8-μm silver filters and measured with a Perkin–Elmer model 240 B elemental analyzer. Chlorophyll *a* was collected on Gelman Type AE glass fiber filters and measured fluorometrically (Yentsch and Menzel 1963).

The measurement of both SPM and POC included a contribution from phytoplankton. As phytoplankton cells are generally not colonized by bacteria (Droop and Elson 1966; Kogure et al. 1982), we wanted to partition phytoplankton biomass from these measures. We assumed a C:chlorophyll *a* ratio of 24 and a dry weight:C ratio of 3.33. The C:chlorophyll *a* ratio was derived from measurements of suspended particulate volume and chlorophyll *a* made on a cruise in the Bay of Fundy during summer 1978 (K. Kranck, Atlantic Oceanographic Laboratory, Bedford Institute of Oceanography, personal communication). There was a consistent peak in volume of particles between 20 and 40-μm diameter which was attributable to phytoplankton. When the peak volume was plotted against chlorophyll *a* for all samples, a volume:chlorophyll *a* ratio of 0.62 was calculated for the peak; with that ratio, the geometric mean cell size (28.3 μm), and the relation between carbon $cell^{-1}$ and cell volume from Strathmann (1967), we calculated a factor of 24 to convert from chlorophyll *a* to carbon for the phytoplankton in this peak. To convert from cell carbon to dry weight we assumed that ash content was 40% (range of 8–57%, Parsons et al. 1961), and that carbon accounted for 50% of the total organic matter. The values of SPM and POC were adjusted to eliminate the contribution of phytoplankton with these factors and the measured value of chlorophyll *a* at each station. Throughout this paper we refer to these as detrital POC and detrital SPM to indicate that the phytoplankton are not included.

Bacteria Numbers and Biomass

One (spring) or two (summer, fall) 10-mL bacteria samples were taken from each bottle, preserved with the addition of 0.5 mL 0.2-μm filtered formalin, and kept refrigerated. The samples were stained with acridine orange and filtered through 0.2-μm Nuclepore filters which were then counted using epifluorescent illumination (Hobbie et al. 1977). Ten (summer, fall) or 20 (spring) fields were counted for each

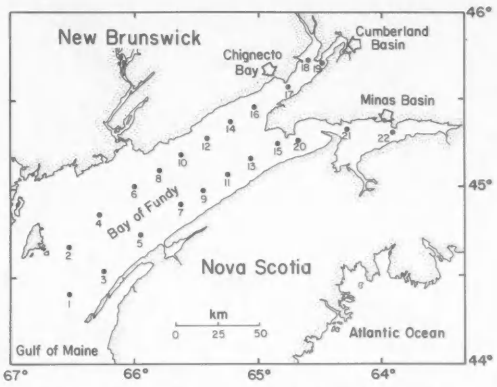

FIG. 1. Location of cruise stations in the Bay of Fundy.

filter and the volume filtered was varied to give about 20 cells per field. Cells were classified as either free-living or attached; attached cells were either on or associated with particulate matter. Kirchman and Mitchell (1982) found that direct counts could underestimate attached bacteria, but we found that blending at high speed for 1 min to break up particles failed to increase significantly the total bacterial cell count for a sample from the spring cruise. In addition to the routine counts, additional counts were made of bacteria retained by 3.0-μm Nuclepore filters for some of the stations where heterotrophic activity was measured. Average cell sizes were determined for some of the stations by observing 100 cells and placing them in one of seven categories; cocci ≤0.5 μm in diameter (assumed volume of 0.065 μm^3) and 0.5–1.0 μm (0.524 μm^3); rods ≤0.5 μm in length (0.035 μm^3), 0.5–1.0 μm (0.126 μm^3), 1.0–1.5 μm (0.188 μm^3), and 1.5–2.0 μm (0.251 μm^3); volumes were individually calculated for other cells. Biomass was estimated from cell volume by assuming a cell density of 1.1 $g \cdot cm^{-3}$, a dry:wet weight ratio of 0.23, and a carbon content of 0.344 (Ferguson and Rublee 1976).

HETEROTROPHIC ACTIVITY

Heterotrophic activity was measured for surface water only during the summer cruise using [^{3}H]glutamic acid (L-[3, 4-^{3}H]-; New England Nuclear) and the kinetic approach of Wright and Hobbie (1965). The labeled glutamic acid was diluted with unlabeled glutamic acid in 0.2-μm filtered seawater so that five levels of added substrate could be used (0.07, 1.07, 2.07, 3.07, and 4.07 $\mu g \cdot L^{-1}$) each with 39.6 $kBq \cdot mL^{-1}$. The incubations were carried out for 1 h at ambient temperature in a shaker in the dark; uptake was linear for both free-living and attached bacteria during this time. We used 25-mL Erlenmeyer flasks, each with 10 mL of sample and 0.2 mL diluted glutamic acid (7.8 kBq). There were duplicate live flasks for each level, although for some stations four replicates were run at the lowest level. Single blanks, killed initially by addition of 0.5 mL formalin, were used for the lowest and highest levels, only. At the end of an hour, all live flasks were killed by addition of 0.5 mL formalin; preliminary studies showed that this method of stopping the incubation gave higher uptake than diluting with concentrated unlabeled glutamic acid, immediate filtration, or killing with H_2SO_4. The contents of each flask were filtered through 0.2-μm Nuclepore filters which were then rinsed with 10 mL of an ice-cold, glutamic acid–seawater solution (10 $mg \cdot L^{-1}$). The filters were placed in 10 mL Aquasol-2 and counted by liquid scintillation. To distinguish uptake due to bacteria attached to particles from that due to free-living bacteria, an additional set of samples was used at the lowest level of addition and treated identically with the first set except that filtration was with 3.0-μm Nuclepore filters; these filters should retain only those bacteria associated with particles if a suitable washing procedure is used (Hodson et al. 1981b).

During the fall cruise we chose to measure only turnover time of the glutamic acid using the single concentration method (Gocke 1977) so that we could investigate variation of heterotrophic activity with depth throughout the bay. We used an addition of 0.09 $\mu g \cdot L^{-1}$ (37 $kBq \cdot L^{-1}$) glutamic acid with four live and two killed flasks for each depth (surface, mid-, and bottom). At the end of the incubations, two of the live flasks and one of the blanks for each depth were filtered through 0.2-μm filters and the other flasks were filtered through 3.0-μm filters.

We also carried out two other types of heterotrophic uptake experiments. During the summer cruise we investigated uptake over time to ensure that the uptake was linear over the 1st hour for both attached and free-living bacteria. There were two of these experiments (time-course I and II), one at the lowest level of added glutamic acid (0.07 $\mu g \cdot L^{-1}$) and one at the highest (4.07 $\mu g \cdot L^{-1}$); surface water from near station 21 was used for the low level and from station 22 for the high level. We incubated a total of 36 flasks and killed four by addition of 0.5 mL formalin after each of the following time periods: 0, 5, 10, 15, 20, 30, 45, 60, and 90 min. For each time interval, the contents of two of the formalin-killed flasks were filtered through 0.2-μm filters and the contents of the other two through 3.0-μm filters; in addition, we counted attached and free-living bacteria at each time interval. The second variation of the uptake studies was to examine attached vs. free-living uptake at all levels of addition during the kinetic experiments, not just at the lowest level. These experiments used two live and one killed flask for each filter size (0.2 and 3.0 μm) at each level, or a total of 30 flasks. We carried out these experiments three times, once on September 11, 1979 using water obtained along the shoreline near station 19, and twice during the fall cruise at stations 17 and 18.

To compare the activity of free-living and attached bacteria, we computed the uptake per cell for both the 0.2-μm and 3.0-μm filters. Free-living bacterial abundance and uptake was determined by subtracting the 3.0-μm values from those determined with the 0.2-μm filters. We also computed the maximum uptake velocity (V_{max})$\cdot cell^{-1}$ for the summer samples as suggested by Wright (1978). To facilitate comparison of $V_{max} \cdot cell^{-1}$ among stations, we used the Arrhenius equation to transform the values of V_{max} to those at the mean temperature for the stations measured. This equation is

$$\text{Ln}\,(V_1/V_2) = (\mu/2) \cdot \{(1/T_1) - (1/T_2)\}$$

where V_1 is the uptake rate at absolute temperature T_1, V_2 is the velocity at T_2, 2 is the approximate value of the gas constant, and μ is the activation energy. We used the value of 21 900 cal (1 cal = 4.1868 J) for μ found by Takahashi and Ichimura (1971) for surface water in a mixed region of the Pacific Ocean.

Results

Abundance and Distribution of Bacteria

Bacterial abundance in surface water of the Bay of Fundy ranged from 0.2 to 4.0 × 10^6 cells·mL^{-1} (Fig. 2). Distribution of free-living bacteria was fairly uniform within the lower Bay of Fundy with the exception of stations 2–6 during the summer where numbers were higher; station 6, located just off Saint John, New Brunswick, had the highest numbers of free-living bacteria recorded during the three cruises. In general, free-living bacteria were most abundant in the summer and least abundant in the spring. Abundance was reduced in the upper reaches of the Bay (Chignecto Bay and Minas Basin) during all three seasons. The distribution of attached bacteria was also quite uniform in surface water during summer and fall in the lower Bay. In the spring, however, there was a marked discrepancy between the northern and southern sides of the Bay, with higher numbers on the northern side except near the mouth of the Bay. In all three seasons the numbers of attached bacteria were much higher in the upper reaches than in the lower Bay: 67–94% of the cells in the upper reaches were attached in the spring, 8–40% in the summer, and 37–89% in the fall.

The abundance of bacteria in the midwater and bottom water of the Bay generally followed that of the surface water (Fig. 2). The major exception was in the summer when high numbers of free-living bacteria in the surface water of stations 2–6 were not reflected in the deeper waters. As in the surface waters, free-living bacteria were generally more abundant in the summer than in the spring and fall and in the lower Bay than in the upper reaches. Attached bacterial numbers were higher along the north side of the Bay than along the south side in the summer in both mid- and bottom-water.

Both the abundance of attached bacteria and the proportion they made up of the total bacterial population were strongly correlated with the amount of detrital SPM. The relation between numbers of attached bacteria and SPM was consistent regardless of season or depth (Fig. 3, $r^2 = 0.94$, $N = 116$, $P < 0.01$); numbers from station 19 in the fall were exceptionally high and were not included in the regression calculation. The relation between the percentage of attached cells and detrital SPM levels was similar in the summer and fall, but in spring a greater fraction of the cells were attached for a given detrital SPM level (Fig. 4); this difference was due to reduced numbers of free-living bacteria in the spring compared to the summer or fall (Fig. 2), not greater numbers of attached bacteria. As one measure of the effect of quality of the detrital SPM on bacterial attachment, we calculated the correlation between attached bacteria (g detrital SPM)$^{-1}$ and the percent carbon of the detrital SPM; r^2 was 0.13 ($N = 110$, $P < 0.05$) indicating only a minor effect. Although the number of attached bacteria per unit weight of detrital SPM was highly variable for detrital SPM concentrations less than 15 mg·L^{-1} ($N = 94$, Fig. 3), ranging from 0.3 to 13.7 × 10^{10} cells (g detrital SPM)$^{-1}$, the variability was much less at higher detrital SPM concentrations. For samples with 15–70 mg detrital SPM·L^{-1} ($N = 17$), the numbers of attached cells (g detrital SPM)$^{-1}$ were between 1 and 2 × 10^{10}, and for detrital SPM concentrations greater than 75 mg·L^{-1} ($N = 8$), there were consistently 1 × 10^{10} cells (g detrital SPM)$^{-1}$.

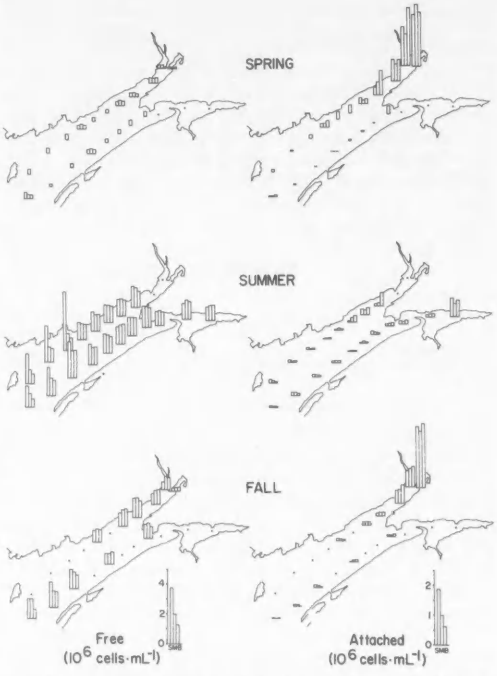

FIG. 2. Abundance of free-living and attached suspended bacteria (× 10^6·mL^{-1}) in the Bay of Fundy. Only one bar is present when only surface water was sampled.

There was no significant difference in bacterial cell volume between spring and summer or with depth in the summer. The mean cell volume for surface water from four stations in the spring was 0.11 μm^3 (SE ± 0.02), equivalent to about 1.0 × 10^{-14} g C·$cell^{-1}$. For five summer stations, overall mean cell volume was 0.09 μm^3 (SE ± 0.01), equivalent to 0.8 × 10^{-14} g C·$cell^{-1}$. Mean cell volumes (μm^3) for surface, mid-, and bottom-water for these five stations were 0.08 ± 0.01, 0.09 ± 0.01, and 0.09 ± 0.02, respectively.

Attached and free-living cells were similar in size during summer. A comparison at station 20, where 30–49% of the total cells were attached, gave cell volumes as follows (μm^3, attached, free-living): surface, 0.08, 0.07; midwater, 0.16, 0.11; bottom, 0.11, 0.11. In addition we analyzed all summer stations for which we determined cell volume (five stations, three depths) to see if cell volume was a function of the percentage of attached cells; this analysis assumes that any

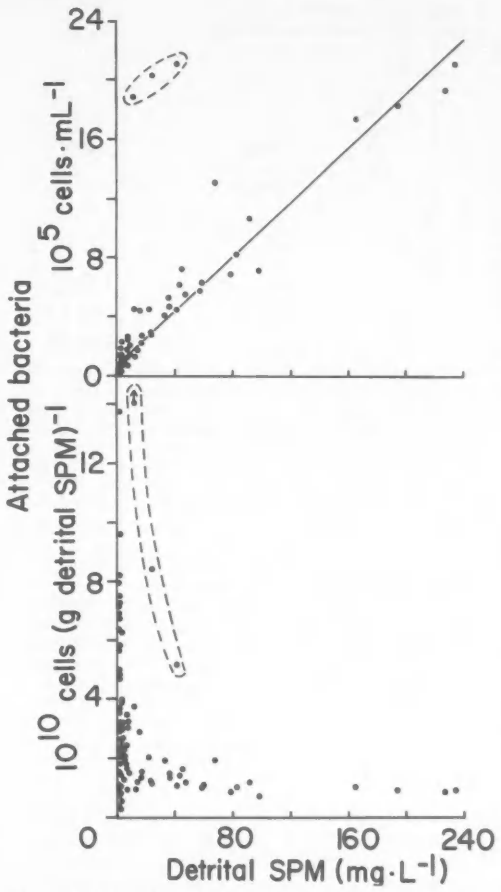

FIG. 3. Total numbers of attached bacteria (*upper*) and number of attached bacteria per unit SPM (*lower*) as a function of SPM concentration for all samples. Values within broken lines are from station 19 on the fall cruise.

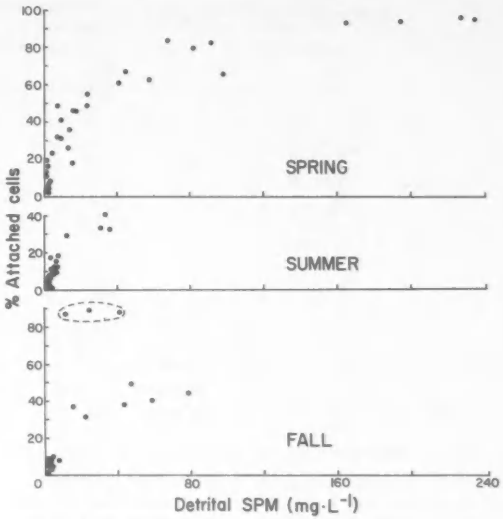

FIG. 4. Percentage of suspended bacteria that were attached as a function of SPM concentration for each season.

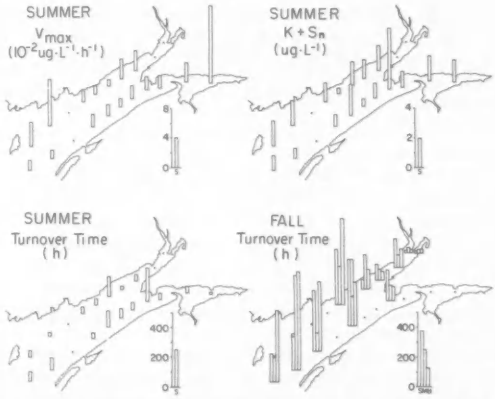

FIG. 5. Turnover time, maximum uptake velocity (V_{max}), and the sum of the half-saturation constant and the ambient substrate concentration ($K + S_n$) for summer and fall.

size difference between free-living and attached cells would be similar throughout the Bay. Regression analysis showed no apparent dependence of cell volume on percentage of attached cells ($r^2 = 0.11$).

HETEROTROPHIC ACTIVITY

Turnover times for glutamic acid were generally higher in the fall than in the summer (Fig. 5). Summer turnover times ranged from 13 to 224 h (mean, 64) while in fall, turnover times were surface, 17–445 h (mean, 205); midwater, 25–631 h (mean, 198); and bottom, 21–658 h (mean, 267). Lowest turnover times were in the uppermost reaches of the Bay.

The maximum uptake velocity (V_{max}) in summer (Fig. 5) was similar throughout the Bay, ranging from 0.01 to 0.03 $\mu g \cdot L^{-1} \cdot h^{-1}$, with two exceptions. The highest V_{max} (0.10) was for station 22 in Minas Basin where the highest concentration of attached bacteria was found. A second high V_{max} (0.06) was found at station 4, just seaward of Saint John.

A multiple regression model using the abundance of free-living and attached bacteria as independent variables explained most of the variation in V_{max} ($R^2 = 0.82$), but very little in either $K + S_n$ (the sum of the half-saturation constant K and the ambient substrate concentration S_n; $R^2 = 0.03$) or turnover time ($R^2 = 0.09$ in summer, $R^2 = 0.31$ in fall). The addition of DOC, chlorophyll, and detrital SPM

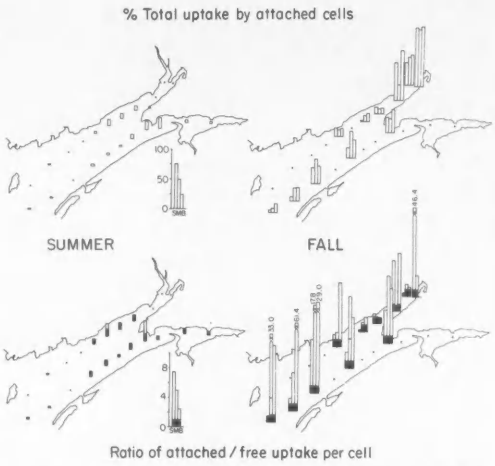

FIG. 6. Percentage of total uptake due to attached bacteria and ratio of attached/free-iiving uptake per cell during summer and fall. Ratio histograms are solid below 1.0 to indicate the point where attached cells become more active than free-living cells.

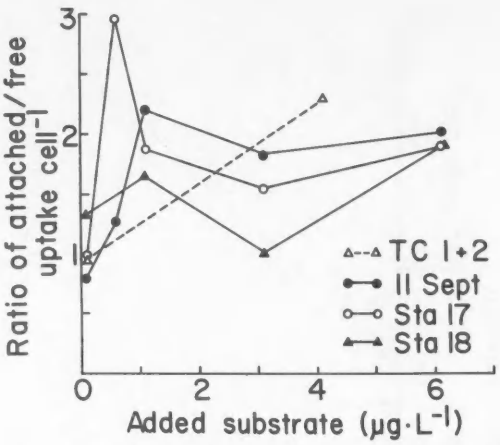

FIG. 7. Ratio of attached/free-living uptake per cell as a function of substrate concentration for the time-course experiments (TC 1+2) and for experiments carried out on September 11 and during the fall cruise at stations 17 and 18.

TABLE 1. Calculation of activity per bacterial cell during the two time-course uptake experiments. Linear regressions were calculated for uptake (DPM) vs. time for the free and attached bacteria; r^2 for the regressions is given along with the slopes (DPM · min^{-1}). The slopes divided by the number of bacteria in the vials gives the uptake cell^{-1} in DPM · min^{-1}. Comparison of uptake per cell is meaningful only within each experiment, not between the two, as natural substrate levels were not determined. (*Significant at the 0.05 level.)

Experiment	Bacteria	r^2	Total uptake (DPM · min^{-1})	Total bacteria ($\times 10^5$)	Uptake per cell (DPM · min^{-1} $\times 10^{-5}$)
Time-course I	Free	0.98*	100.6	84.5	1.19
(low level of substrate)	Attached	0.99*	8.6	7.5	1.14
Time-course II	Free	0.95*	49.6	79.4	0.62
(high level of substrate)	Attached	0.98*	87.5	61.1	1.43

concentrations to the model improved the fit significantly ($P < 0.01$) only for V_{max} ($R^2 = 0.94$).

Activity of attached bacteria differed from that of free-living bacteria. The percentage of the total uptake due to attached cells showed a marked increase in the upper reaches of the Bay in the fall (Fig. 6) and, in general, was higher in the fall than in the summer. It was possible to calculate the relative activity of attached and free-living bacteria on a per cell basis by taking into account both the percentage of attached bacteria and their contribution to the total activity (Fig. 6). There did not appear to be any apparent trend throughout the Bay or with depth, but in general attached cells were less active than free-living cells in the summer and more active in the fall.

The relative activity of attached cells appeared to increase with substrate level. The two time-course experiments (Table 1; Fig. 7) showed no difference in activity per cell between attached and free-living bacteria at the low substrate level, but over a two-fold greater activity for attached cells at the high substrate level. The three experiments where a full range of substrate levels was used (Table 2, 3; Fig. 7) also showed the same trend. A comparison of calculated values of $V_{max} \cdot \text{cell}^{-1}$ (Table 3) showed that in each case attached cells were potentially more active than free-living cells. However, the actual ratio of attached to free-living activity was always less than the ratios of V_{max}. The calculated values of $K + S_n$ (Table 2) show that the half-saturation constant K of attached cells was greater than that for free-living cells in each of the experiments because the ambient substrate concentration S_n was the same for both cell types. Thus, the free-living bacteria should have been better able to use the low natural substrate levels than the attached cells, and this was what we observed.

By assuming that bacteria throughout the Bay differed mainly in attachment to particles, it was possible to calculate $V_{max} \cdot \text{cell}^{-1}$ for attached and free-living bacteria in summer surface water (Table 3). Using the Arrhenius equation to correct V_{max} for temperature differences between stations (see Methods and Materials), we fitted the data to a multiple regression equation with the constraint that the constant would be 0:

TABLE 2. Results of three kinetic experiments carried out with water obtained along the shoreline near station 19 on September 11, 1979, and during the fall cruise at stations 17 and 18. One data point was omitted before calculating the September 11 free regression. (*Significant at the 0.05 level.)

Experiment	Bacteria	Cell numbers ($\times 10^5 \cdot mL^{-1}$)	V_{max} ($\mu g \cdot L^{-1} \cdot h^{-1}$)	$K + S_n$ ($\mu g \cdot L^{-1}$)	r^2
September 11 shoreline	Free	6.10	0.033	2.3	0.96*
	Attached	10.40	0.204	8.4	0.96*
Station 17, fall cruise	Free	9.52	0.025	3.4	0.93*
	Attached	2.39	0.014	5.0	0.90*
Station 18, fall cruise	Free	5.09	0.015	1.4	0.78*
	Attached	5.95	0.040	4.0	0.92*

TABLE 3. Calculation of $V_{max} \cdot cell^{-1}$ from the data in Table 2 and from a regression for all summer surface stations. Values for the ratio of the actual uptake rate (v) of attached cells to free-living cells are from Fig. 7; this ratio was not calculated for the summer surface stations as it would have required the assumption that S_n was constant throughout the Bay.

Experiment	$V_{max} \cdot cell^{-1}$ ($\mu g \cdot h^{-1} \times 10^{-11}$) Attached	Free	V_{max} A/F	v A/F
September 11	19.58	5.41	3.62	0.79
Station 17, fall	5.86	2.63	2.23	0.99
Station 18, fall	6.72	2.95	2.28	1.33
All summer surface stations	8.71	1.07	8.14	--

$$V_{max} = 0.00107 \cdot F + 0.00871 \cdot A \ (R^2 = 0.90, \ N = 17, \ P < 0.01)$$

where V_{max} is $\mu g \cdot L^{-1} \cdot h^{-1}$, F is free-living bacteria, and A is attached bacteria (both $\times 10^8 \cdot L^{-1}$); the coefficients of F and A represent V_{max} $(10^8 \text{ cells})^{-1}$. Therefore, $V_{max} \cdot cell^{-1}$ for attached bacteria was greater than that for free-living bacteria, in contrast to the actual uptake $cell^{-1}$ which was generally higher for free-living bacteria during the summer (Fig. 6).

Discussion

The proportion of suspended bacteria that are attached appears to depend on the area sampled. Hanson and Wiebe (1977) point out that in the open ocean most bacteria are free-living, in coastal waters the proportion of attachment appears to vary, and in estuary–salt marsh systems most of the bacteria seem to be attached. The Bay of Fundy apparently has similarities to all these systems with the relatively open mouth, adjacent to the Gulf of Maine, dominated by free-living bacteria, and the turbid upper reaches dominated by attached cells. Kirchman and Mitchell (1982) suggested a classification of waters into two types: one group has salinities less than 10‰, tidal flushing does not occur regularly, abundance of suspended particles is relatively high, and attached bacteria are relatively important; the second group has higher salinity, greater tidal flushing, and fewer suspended particles, and attached bacteria are relatively less important. The Bay of Fundy does not fit well in this classification scheme because high salinity (generally 27–33‰) and tidal flushing are present along with high suspended particle load and a relatively important attached bacterial population. The abundance of SPM seems to be the controlling factor in the Bay (Fig. 4), and this may also be the case in other areas.

Both the distribution and activity of suspended bacteria in the Bay of Fundy reflect the general anticlockwise circulation pattern of flow in from the Gulf of Maine along the southern or Nova Scotian coast and out along the northern New Brunswick coast (Bailey 1957). For example, the distribution of free-living bacteria during the summer (Fig. 2) shows an initial stratification as water enters the Bay along the southern coast followed by relatively complete mixing so that distribution becomes uniform with depth. As the water flows out along the northern coast, the distribution remains uniform with depth until past the Saint John River outflow where a pulse of bacteria appears in the surface water; this pulse remains evident in the more seaward stations. Similarly, the distribution of attached bacteria in the spring shows the influence of SPM carried out of Chignecto Bay along the northern coast. Thus, in some respects, bacteria appear to be a conservative tracer of water masses in this study.

The upper reaches of the Bay show a higher degree of heterotrophic activity as well as greater numbers of suspended bacteria than the lower Bay, presumably because of the abundance of SPM. Water samples taken from shallow estuaries entering Cumberland Basin commonly had SPM levels from 5 to 20 $g \cdot L^{-1}$ (D. C. Gordon, Marine Ecology Laboratory, Bedford Institute of Oceanography, personal communication), and corresponding bacterial counts were as high as 10^8 cells $\cdot mL^{-1}$ with 96% of the cells attached (L. M. Cammen and J. A. Walker unpublished data). Most of the heterotrophic activity in the upper reaches of the Bay was due to attached bacteria during the fall (Fig. 6). This may also have been true in spring, but we cannot be certain as no activity measurements were made then.

A general description of summer and fall heterotrophic activity in the Bay would be as follows: activity was greater in the summer than in the fall (based on turnover times) and was dominated by free-living bacteria throughout the Bay. In the fall, free-living bacteria still accounted for most of the heterotrophic activity in the lower Bay but the proportion of

activity due to attached bacteria was substantially greater than during the summer. Activity in the upper reaches in the fall was dominated by attached bacteria. On a per cell basis, the geometric mean ratio of attached:free-living activity in surface water was 0.56 in the summer and 2.32 in the fall. Thus, free-living bacterial cells were about twice as active as attached cells in surface water during the summer, but only half as active during the fall.

Several qualifications of this general description are necessary. First, the comparison of heterotrophic activity between summer and fall is based mainly on turnover times which are a function not only of substrate uptake rates but also of substrate concentrations in the water. Although no measurements of glutamic acid were made during this study, amino acid concentrations in an estuary of the Gulf of Maine (Keller et al. 1982) and in a North Carolina estuary (Crawford et al. 1974) were similar in August and late October–early November, the time of our summer and fall cruises. Without actual measurements of glutamic acid concentrations in the Bay, it is not possible to be certain that the increase in turnover time in the fall represents an actual decrease in activity, although the studies in other estuaries tend to support this view.

A second necessary qualification for the seasonal comparison of suspended bacteria is the fact that Minas Basin was sampled during summer, but not fall, and Chignecto Bay was sampled during fall, but not summer. Samples taken concurrently with the summer cruise in Cumberland Basin, at the location of station 19, gave numbers of free-living bacteria similar to those in Minas Basin, but attached bacteria counts were much higher than in Minas Basin, and in fact made up 56% of the total cell count (L. M. Cammen and J. A. Walker unpublished data). This suggests that a substantial portion of the total summer heterotrophic activity in Cumberland Basin might be due to attached cells. Thus, there appears to be a substantial difference between the two upper basins of the bay in summer, but there are no comparative samples for the spring and fall.

The third qualification to these data is that we have assumed that all uptake of glutamic acid was due to bacteria, particularly in our comparisons of free-living and attached activity per cell. Of course, there are other organisms such as microalgae which take up glutamic acid. However, at natural substrate levels most of the heterotrophic uptake is generally due to bacteria (Hanson and Wiebe 1977; Riemann 1978).

Our data on the relative activity per cell of attached and free-living bacteria can be summarized by these observations:

1) At natural substrate levels in the summer, free-living cells were twice as active on a per cell basis as attached cells, but in the fall they were only half as active. Although we measured only the glutamic acid incorporated by the bacteria with no correction for respiration, the comparison between free-living and attached cells is still valid as they should have respired a similar fraction of the assimilated substrate (Hanson and Wiebe 1977). There was no difference in cell volumes between free-living and attached cells which could explain the difference in activity as was the case for the data of Hodson et al. (1981b). Kirchman and Mitchell (1982) also found that cell volumes could not explain activity differences.

2) During both summer and fall an increase in substrate level resulted in a greater increase in activity of attached cells than of free-living cells. This finding apparently differs from that of Azam and Hodson (1977) and Riemann (1978), who found no such effect. However, fig. 8 from Azam and Hodson (1977, p. 499) shows that in one of their two experiments a similar effect was present; in terms of total uptake the change was small (about 93–94% uptake by free-living cells at low concentration compared to 79–80% uptake at high concentration), but in terms of the ratio of free-living to attached uptake this change is significant, representing almost a fourfold increase in the relative activity of attached cells compared with free-living cells at the highest concentration. It appears that although this effect may not always be present, it is not unique to our samples.

3) During both summer and fall $V_{max} \cdot cell^{-1}$ was higher for attached cells than for free-living cells. There are several possible explanations for the differences we observed in activity between free-living and attached cells. We have assumed, without testing, that the specific activity of the assimilated glutamic acid was similar for both the free-living and attached bacteria; this assumption seems reasonable, but if it were not correct, then our uptake measurements would have been affected. It is also possible that at low, natural, substrate concentrations the only organisms in the >3.0-μm fraction capable of significant activity are the attached bacteria, but as the substrate concentration increases, the other organisms in this fraction (microalgae, for example) increase their activity and thus the fraction of total uptake due to >3.0-μm organisms increases. This effect, however, is unlikely at even the highest substrate levels that we used (6.09 $\mu g \cdot L^{-1}$) as K values for algae are in the range of hundreds of micrograms$\cdot L^{-1}$ (Wright and Hobbie 1966). A more likely interpretation of the data is that attached bacteria are less efficient than free-living bacteria at using low levels of substrate, but more efficient at high levels. The normal environment of free-living cells should be poorer than that of a cell living on a particle which provides a surface for adsorption of DOC and micronutrients (Paerl and Merkel 1982), and it would thus be an advantage for free-living cells to have relatively low values of K (Table 2).

It appears then from our data that communities of free-living bacteria in the Bay of Fundy have relatively low K and V_{max} while communities of attached bacteria have relatively high K and V_{max}. We have no way of determining from our data whether the variation in activity between attached and free-living bacteria represents a response of individual cells or a community response. It is possible that instead of variation in the individual activity per cell, we are seeing a change in the percent of active cells (Bell and Albright 1982). It may be that the differences are a result of assemblages of multiple species of bacteria, all with differing K and V_{max} (Williams 1973). To assess these possibilities, it will be necessary to use techniques capable of determining whether an individual cell is active, such as incubation with INT (Zimmerman et al. 1978) or autoradiography (Hoppe 1976). Whatever the underlying mechanism, though, the difference in the responses of free-living and attached bacteria to increased substrate is important, because the ratio of free-living:attached activity will necessarily vary with varying substrate concentrations. This may be one explanation for the seasonal variation observed in this study as well as the contradictory results for other studies.

Acknowledgments

We thank the following for allowing us to use their unpublished data: Carl Amos (SPM, water temperature), Donald Gordon (POC, Chl *a*, SPM from estuaries), and Kate Kranck (SPM volume and Chl *a* from a previous cruise). This manuscript benefited from reviews by Donald Gordon, David Kirchman, and Parke Rublee. Support for this study was through a National Research Council Canada Visiting Fellowship (L.M.C.) and the Canadian Job Corps (J.A.W.).

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Meristic variation in beaked redfishes, *Sebastes mentella* and *S. fasciatus*, in the Northwest Atlantic

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The distribution of Northwest Atlantic beaked redfishes, *Sebastes mentella* and *S. fasciatus*, has been confused for the past several decades. *Sebastes fasciatus* has been reported as having lower meristic counts than *S. mentella*. Meristic elements of 33 301 vertebrae, 22 622 anal fin ray, and 16 290 dorsal fin ray counts were utilized to examine the yearly, depth, and geographic variation of meristics in beaked redfishes. Data collected from specimens caught off West Greenland, Baffin Island, Labrador, Newfoundland, Quebec, and Nova Scotia were analyzed by 100-m-depth intervals. Temporal and depth variation were examined by the χ^2-test of independence on meristic frequencies. Geographic variation was evaluated using the modes and mean values in the meristic frequency histograms. Cluster analysis of meristic frequencies displayed the dendrographic affinities and the distance matrix among division–depth blocks.

Temporal variation indicated a mixture of vertebral frequency patterns which varied with depth and area. Depth variation and geographic clines were found for each of these meristic characters; the shallow and southern waters were dominated by counts of 29, 14–13, and 7 for vertebrae, dorsal fin rays, and anal fin rays, respectively, whereas deep and northern waters were dominated by 30, 15–14, and 8–9. These results suggested that *S. fasciatus* occurs on the Nova Scotian Shelf and Grand Bank whereas *S. mentella* is largely found in Baffin Bay, Labrador waters, and the Gulf of St. Lawrence. The common names of *S. mentella* and *S. fasciatus* are discussed. Additionally, five hypothetical stocks of beaked redfishes based on meristic patterns are proposed for management purposes.

Key words: *Sebastes mentella*, *S. fasciatus*, redfish, meristic variation, distribution, cluster analysis

Ni, I-H. 1982. Meristic variation in beaked redfishes, *Sebastes mentella* and *S. fasciatus*, in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. 39: 1664–1685.

Depuis plusieurs décennies, la distribution des sébastes à bec de l'Atlantique nord-ouest, *Sebastes mentella* et *S. fasciatus*, est confuse. On a rapporté que *S. fasciatus* avait des comptages méristiques inférieurs à ceux de *S. mentella*. Pour une étude de la variation annuelle, bathymétrique et géographique de ces comptages chez ces poissons, les éléments méristiques de 33 301 vertèbres, 22 622 rayons de la nageoire anale et 16 290 rayons de la nageoire dorsale ont été utilisés. Les données recueillies sur des sujets capturés au large du Groenland occidental, de l'île Baffin, du Labrador, de Terre-Neuve, du Québec et de la Nouvelle-Écosse ont été analysées par intervalles de 100 m. La variation temporelle et bathymétrique a été soumise à un test de χ^2 d'indépendance des fréquences méristiques. La variation géographique a été évaluée à l'aide des modes et les valeurs moyennes des histogrammes de fréquences des comptages méristiques. L'analyse des groupements de ces fréquences donne un produit qui se rapproche du dendrogramme et montre la matrice de distance entre blocs d'intervalles de profondeur.

À en juger par la variation temporelle, il y a mélange de distribution des fréquences vertébrales, qui changent selon la profondeur et la région. On constate une variation bathymétrique et des clines géographiques pour chacun de ces caractères méristiques; les eaux peu profondes et méridionales sont dominées par des comptages de 29, 14–13 et 7 pour les vertèbres, les rayons de la nageoire dorsale et ceux de la nageoire anale respectivement, alors que les eaux profondes septentrionales sont dominées par des comptages de 30, 15–14 et 8–9. Ces résultats donnent à penser que *S. fasciatus* se trouve sur le plateau Scotian et le Grand banc, tandis que *S. mentella* se rencontre surtout dans la baie Baffin, les eaux du Labrador et le golfe du Saint-Laurent. Nous examinons les noms vernaculaires de ces deux espèces. De plus, pour des fins de gestion, nous proposons cinq stocks hypothétiques de sébastes à bec sur la base des caractères méristiques.

Received February 18, 1982
Accepted August 18, 1982

Reçu le 18 février 1982
Accepté le 18 août 1982

Printed in Canada (J6823)
Imprimé au Canada (J6823)

AN understanding of the distribution of beaked redfishes, *Sebastes mentella* and *S. fasciatus*, is important for the appropriate design of life history studies of each species and for determining component stocks. However, in past decades this was hampered by the uncertainties of redfish systematics and species identification. This study examines the yearly, depth, and geographic variation in meristic characters, which are used to discriminate between *S. mentella* and *S. fasciatus*, and explores the distribution of these species in the Northwest Atlantic.

Although morphological differences between these two species were discussed by Barsukov (1972), Barsukov and Zakharov (1972), Litvinenko (1974), and Templeman (1980), the overlapping of morphological characters has made it very hard to distinguish beaked redfishes in field studies. From studies of extrinsic gasbladder musculature, Litvinenko (1980) and Ni (1981a) supported the validity of *S. fasciatus*. From the discriminant analysis of morphological characters, Ni (1981b) concluded that anal fin rays and vertebrae were good discriminators between *S. mentella* and *S. fasciatus*. This has been elucidated by numerous researchers (Table 1) who found that *S. mentella* had 30 vertebrae, 8 or 9 anal fin rays, and 14 or 15 dorsal fin rays, whereas *S. fasciatus* had most commonly 29 vertebrae, 7 anal fin rays, and 14 dorsal fin rays.

In this report, vertebrae, anal fin rays, and dorsal fin rays of beaked redfishes were examined individually and collectively. I assumed that vertebrae and anal fin ray frequency distributions dominated by 30 and by 8 or 9, respectively, indicated *S. mentella*, whereas vertebrae 29 and anal fin rays 7 indicated *S. fasciatus*. Thus, a preliminary understanding of the distribution for *S. mentella* and *S. fasciatus* could be acquired from meristic variation.

Materials and Methods

We collected 33 301 vertebrae, 16 315 anal fin ray, and 16 290 dorsal fin ray counts between 1957 and 1968. An additional 6307 anal fin ray counts were gathered between 1974 and 1981 during Canadian research survey cruises. The areas sampled, covering the whole Northwest Atlantic, were NAFO Div. 0-4X (Fig. 1). Sample sites were along the continental slope at bottom depths of 100–750 m. Data were broken down into 100-m-depth intervals, as Barsukov (1972) and Templeman (1976) suggested that *S. mentella* was distributed in deeper waters than *S. fasciatus*. As beaked redfishes were rarely found in shallow waters, data from waters <200 m were combined into one depth zone.

The temporal variation was analyzed for the vertebrae data only, because of the relatively large sample sizes. Vertebral counts excluded the urostyle, in conformity with Templeman and Pitt's (1961) redfish vertebral studies based on data collected from 1947 to 1954.

Particular attention was given to counting the last ray of the anal fin. This ray consists of two elements that were separated at the very base of the fin, which were seated on one basal pterygiophore. The last two fin elements were counted as one ray.

Yearly and depth variation in meristic frequencies were examined separately using a χ^2-test of independence to identify cells (in the frequency table) or patterns of cells that contributed to a departure from independence. The BMDP2F program (Dixon and Brown 1979) was calculated in a stepwise manner. Stepping stops if the probability of the test-of-fit is greater than 0.05. The cells which contributed most to a significant difference could be deduced from the sequence of cells eliminated.

Geographic variation was deduced from the patterns of modes and means in the meristic frequency histograms. I also applied cluster analysis (the BMDP2M program) to describe the dendrographic affinities and the distance matrix among division–depth blocks. The criterion to join clusters is defined as the distance between two cases in the χ^2-test of equality of the two sets of frequencies. Amalgamation uses the distances between centroids as defined by the average values with the clusters.

Results

VERTEBRAE

Vertebral frequencies for division–depth blocks were first broken down by year to study temporal variation. A summary of the results of the χ^2-tests, for influence of year, is shown in Table 2. Frequencies from Div. 2G and 2H were excluded from the table because insufficient data were available. The vertebral frequencies were stable over time in the waters between Greenland and Baffin Island (Subarea 0 and 1). Vertebral frequencies varied yearly in the shallow waters of southern Labrador (<400 m in Div. 2J), but they were stable in deep waters. In northeastern Newfoundland (Div. 3K), a stable pattern was seen only at the 500- to 599-m-depth zone. On the Flemish Cap (Div. 3M), the unstable depth zone was 300–399 m. On the Grand Bank, vertebral frequencies fluctuated yearly in <500-m waters of Div. 3L, in medium depth zones of Div. 3N (200–299 m and 400–499 m), and in deep waters of Div. 30 (>500 m). On St. Pierre Bank (Div. 3P), vertebral frequencies varied annually in the waters deeper than 200 m. In the Gulf of St. Lawrence (Div. 4RST), vertebral frequencies varied yearly in the 200- to 299-m-depth zone. There were occasional fluctuations in the <200-m and the 300- to 399-m-depth zones as no significant differences were found by eliminating one year's data for both depth zones. On the Nova Scotian shelf, the frequencies in Div. 4V showed no yearly variations in the shallow waters (<400 m) although they varied in the deeper waters; Div. 4W and Div. 4X displayed slight differences in yearly vertebral frequencies in the shallow waters and in the deep waters, but they were stable in the medium depth zones (around 200–500 m). In summary, temporal variation varied with different depth zones for different areas. However, a mixture of different types of vertebral frequencies was clearly indicated.

To assess variation by depth for each division, data collected from different years were combined and vertebral percentage frequency distributions were evaluated (Fig. 2). The χ^2-tests of the independence of vertebral frequencies with depth for each NAFO Division are listed in Table 3. Only the specimens collected from Baffin Bay (Subarea 0 and 1) and northern Labrador (Div. 2G) showed no significant differences of their vertebral frequencies with depth. They

TABLE 1. Samples sizes, mean values, and modes of vertebrae, anal fin ray and dorsal fin ray frequencies recorded in the literature for (A) *Sebastes mentella* and (B) *S. fasciatus*. Abbreviations for locality are NAFO Divisions.

Locality	Vertebrae[a]			Anal fin rays			Dorsal fin rays			Author
	N	Mean	Mode	*N*	Mean	Mode	*N*	Mean	Mode	
A. *S. mentella*										
Barents Sea	37	30.14	30	137	8.48	8	80	14.29	14	Barsukov and Zakharov (1972)
Iceland and Greenland	37	30.27	30	42	8.72	9				Barsukov (1972)
Iceland	253	30.17	30	282	8.49	9	34	14.65	15	Barsukov and Zakharov (1972)
West Greenland	146	29.99	30	235	8.53	9	57	14.53	15	Barsukov and Zakharov (1972)
Baffin Island	397	30.02	30	326	8.54	9	153	14.59	15	Barsukov and Zakharov (1972)
2J	202	30.04	30	203	8.61	9				Barsukov and Zakharov (1972)
3K	232	30.03	30	233	8.51	9				Barsukov and Zakharov (1972)
3L	100	30.03	30	100	8.62	8	100	14.67	15	Ni (1981b)
3O	109	30.10	30	297	8.94	9	42	14.61	14	Litvinenko (1974)
3M, 3P	49	30.00	30	48	8.37	8				Barsukov and Zakharov (1972)
Flemish Cap and Banquereau	48	30.02	30	48	8.40	8				Barsukov (1972)
B. *S. fasciatus*										
2J, 3K	228	29.36	29	232[b]	7.63	8	96[b]	13.79	14	Barsukov and Zakharov (1972)
3L	99	29.31	29	100	7.02	7	100	13.62	14	Ni (1981b)
3O	124	29.05	29	124	7.22	7	84	13.63	14	Litvinenko (1974)
3M, 3N, 3O, 3P	455	29.12	29	455	7.27	7	196[c]	13.67	14	Barsukov and Zakharov (1972)
4W, 5Z	447	29.19	29	489	7.22	7	106[d]	13.73	14	Barsukov and Zakharov (1972)
Flemish Cap, Grand Bank, and Banquereau	41	29.19	29	41	7.22	7				Barsukov (1972)

[a]Data were adjusted by excluding urostyle.
[b]From 3K only.
[c]From 3O, 3P only.
[d]From 4W only.

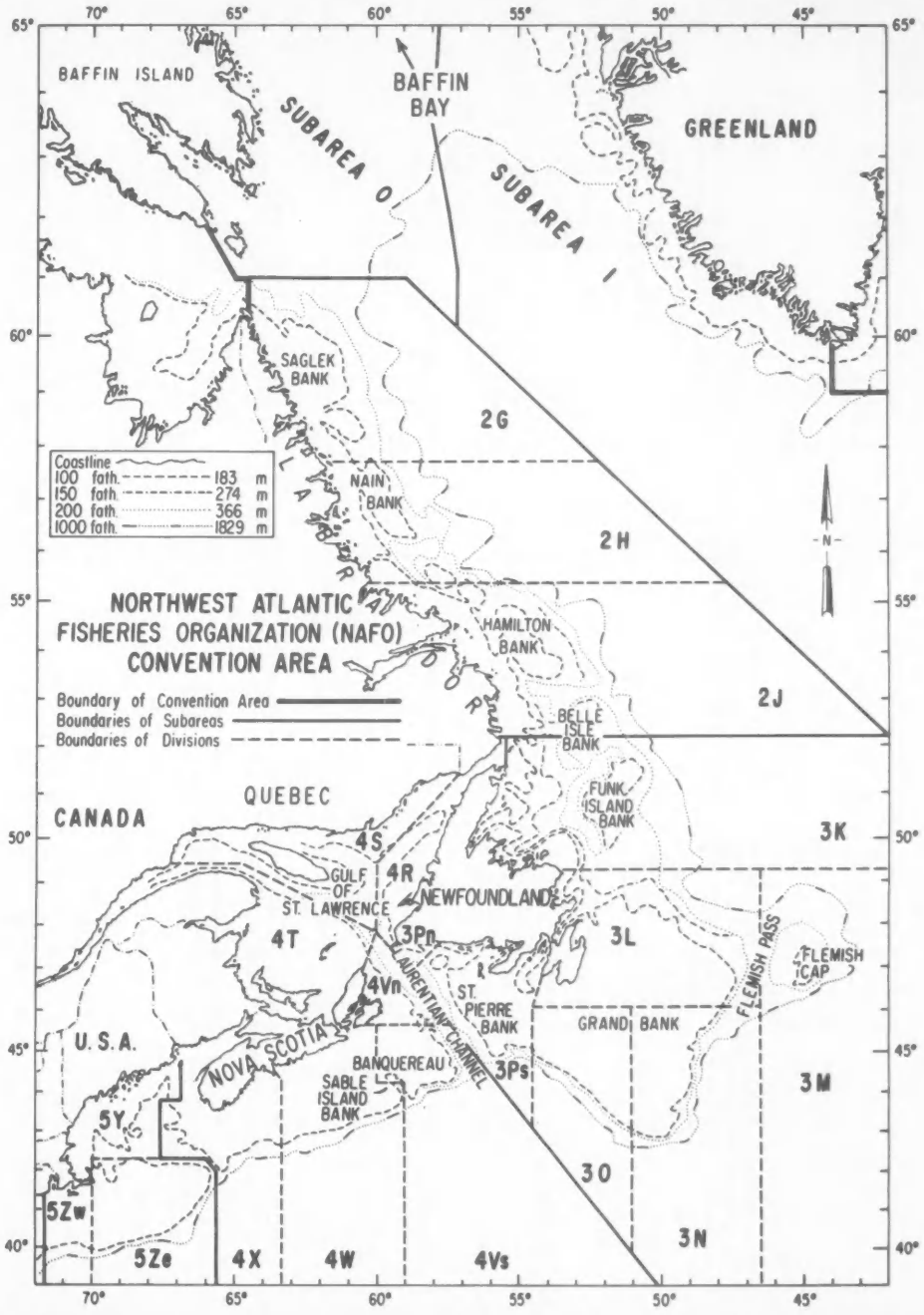

FIG. 1. NAFO Divisions and place names referred to in the text. Sample sites were along the continental slope at bottom depth around 100–750 m.

TABLE 2. Summary of χ^2-test of independence (significant level)[a] for temporal variations of vertebral frequencies in division-depth blocks. Number of years data for calculation are in parentheses. Sample sizes are in Fig. 2. Abbreviations under geographic areas are NAFO Divisions.

Depth range (m)	Baffin Bay		Southern Labrador	NE Nfld.	Flemish Cap	Grand Bank			St. Pierre Bank	Gulf of St. Lawrence			Nova Scotian Shelf		
	0	1	2J	3K	3M	3L	3N	3O	3P	4R	4S	4T	4V	4W	4X
<200							ns	ns	ns	***[b]	*[b]	***[b]	ns	*[b]	*[b]
							(2)	(4)	(8)	(8)	(4)	(6)	(6)	(6)	(3)
200–299			***[b]	*[b]	ns	***	*[b]	ns	***[c]	***	***[c]	***[c]	ns	*[b]	ns
			(4)	(3)	(2)	(4)	(3)	(2)	(8)	(8)	(5)	(6)	(3)	(3)	(3)
300–399	ns	ns	**[b]	***	***	**[b]	ns	ns	***	***[b]	*[b]	ns	ns	ns	*
	(2)	(2)	(3)	(2)	(2)	(4)	(3)	(3)	(4)	(7)	(4)	(5)	(2)	(3)	(2)
400–499	ns	ns	ns	*	ns	**[b]	***	ns		ns	ns	ns	**	ns	ns
	(2)	(2)	(3)	(2)	(2)	(3)	(2)	(3)		(3)	(2)	(2)	(2)	(3)	(4)
500–599		ns	ns	ns	ns	ns	ns	**[b]			—[d]	—		**[b]	*
		(2)	(3)	(2)	(2)	(4)	(2)	(3)						(3)	(2)
≥600		ns	ns	*	***	ns		**	—	—	—	—		ns	*[b]
		(2)	(2)	(2)	(2)	(3)		(2)						(3)	(3)

[a]ns: no significant difference; *: significant difference at $P = 0.05$ level; **: significant difference at $P = 0.01$ level; ***: significant difference at $P = 0.001$ level.

[b]No significant difference was found by eliminating only 1 year data.

[c]No significant difference was found by eliminating 2 years data.

[d]Depth not applicable.

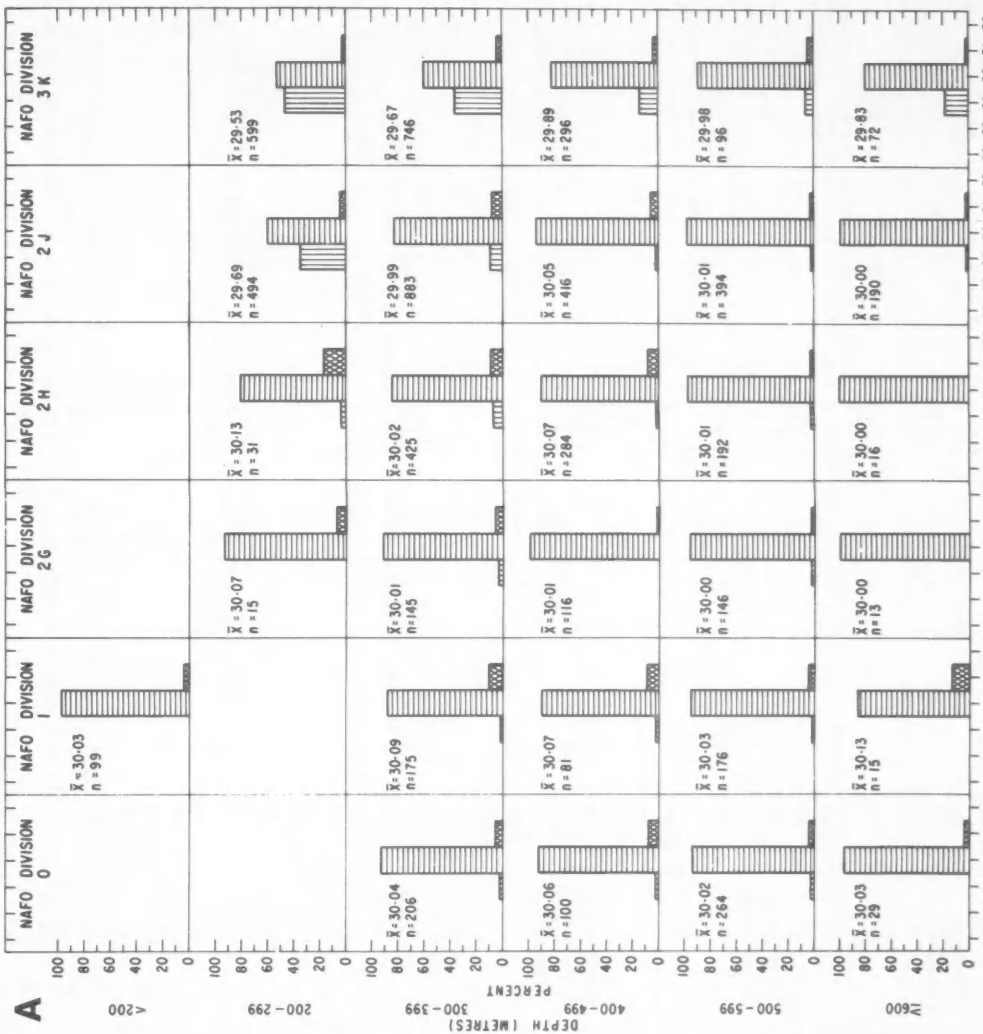

FIG. 2. Percentage frequency histograms of vertebrae in NAFO division-depth blocks.

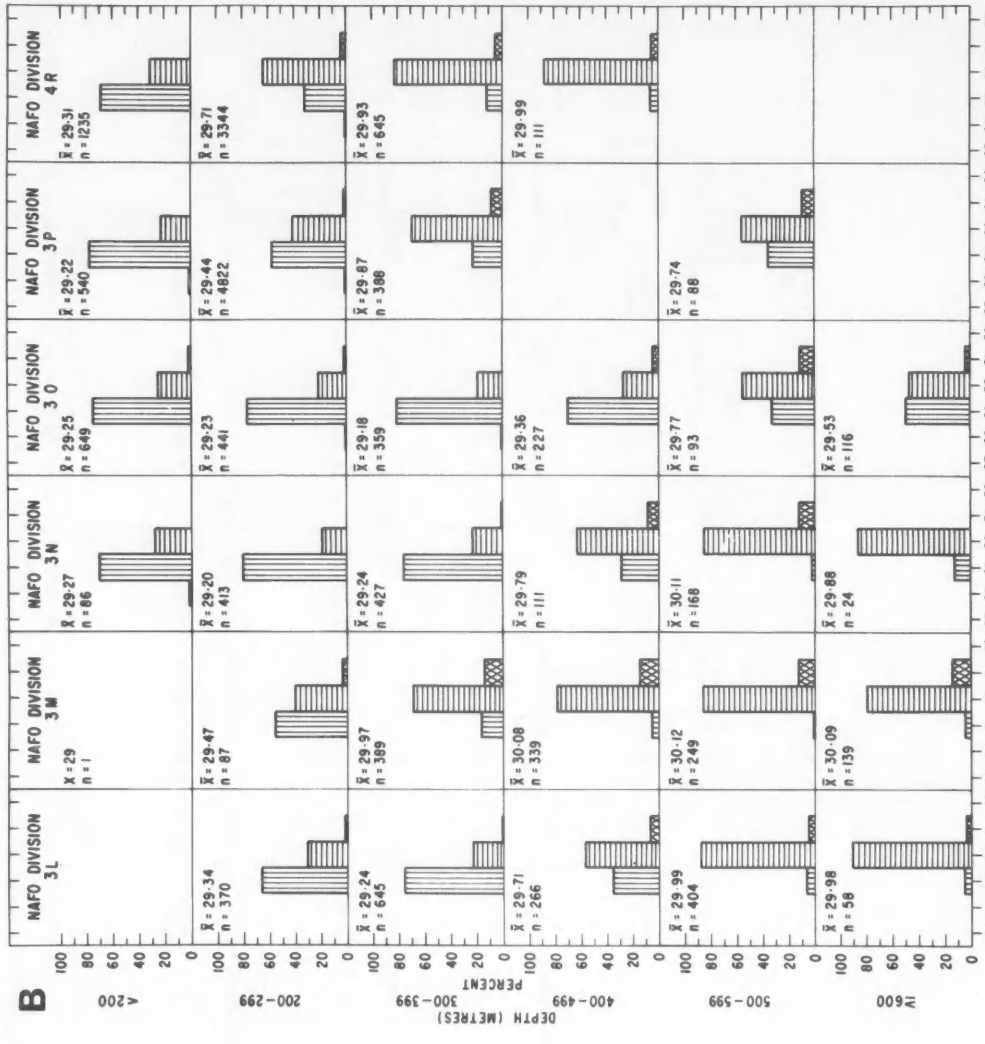

FIG. 2. (*Continued*)

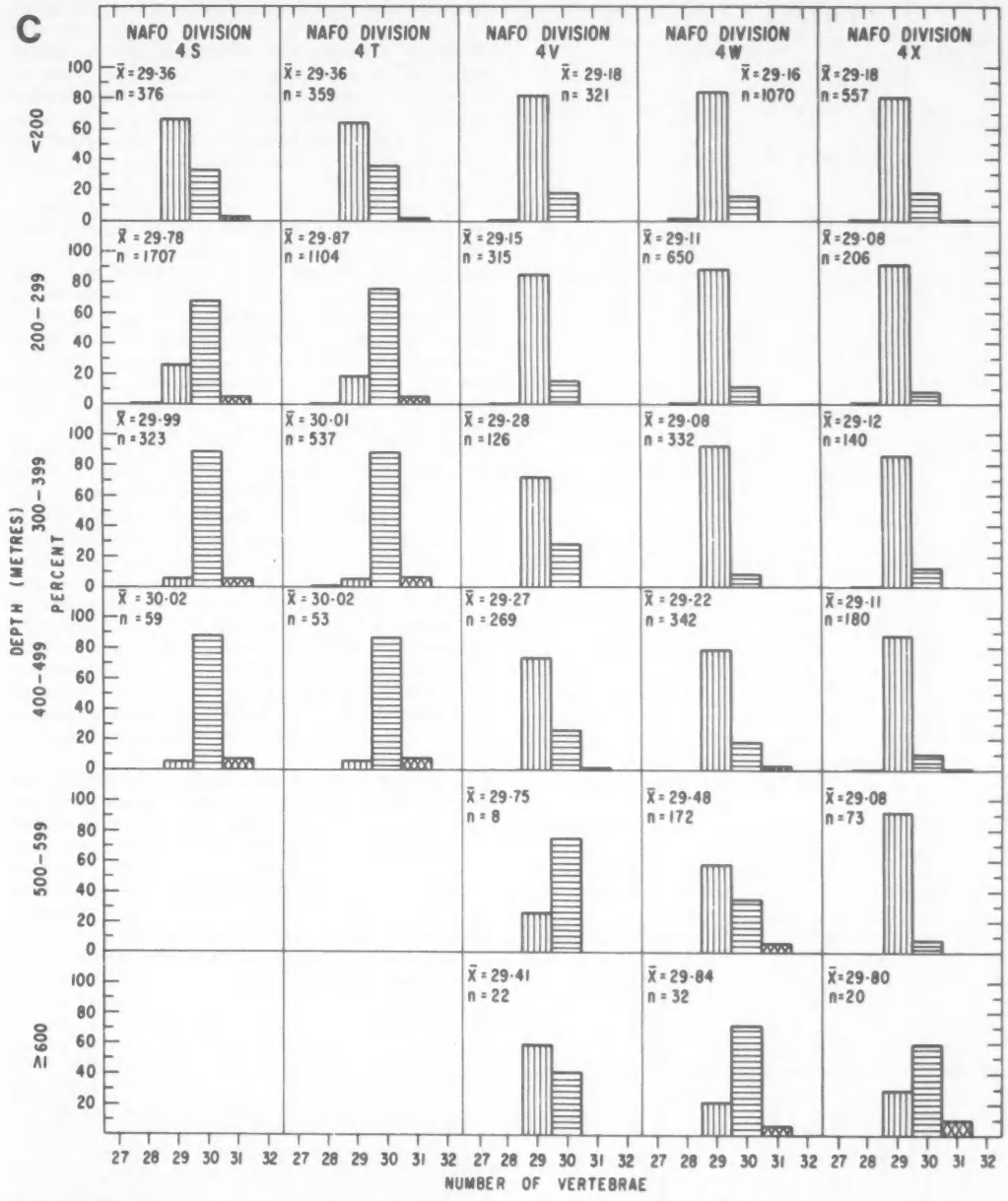

FIG. 2. (*Concluded*)

TABLE 3. The vertebrae statistics of beaked redfishes for each NAFO Division. χ^2-test shows the independence of vertebral frequencies with depth zones used in Fig. 2.

	Baffin Bay		Labrador Shelf			NE Nfld.	Flemish Cap	Grand Bank			St. Pierre Bank	Gulf of St. Lawrence			Nova Scotian Shelf			
Statistics	0	1	2G	2H	2J	3K	3M	3L	3N	3O	3P	4R	4S	4T	4V	4W	4X	Total
N^a	599	546	435	948	2377	1809	1204	1743	1229	1885	5838	5335	2465	2053	1061	2598	1176	33,301
$\bar{x}$	30.03	30.06	30.01	30.04	29.94	29.68	30.01	29.53	29.41	29.29	29.46	29.65	29.75	29.82	29.21	29.17	29.15	29.59
Mode[b]	30	30	30	30	30	30	30	29,30	29,30	29	29,30	30,29	30	30	29	29	29	30,29
Range	29–31	29–31	29–31	29–31	29–31	29–31	29–31	29–31	28–31	28–31	28–31	28–32	28–31	28–31	28–31	27–31	28–31	27–32
$s_{\bar{x}}^2$	0.0104	0.0116	0.0103	0.0106	0.0082	0.0121	0.0145	0.0133	0.0155	0.0112	0.0071	0.0074	0.0106	0.0109	0.0129	0.080	0.0110	0.0033
Percentage of abnormal vertebrae	0.83	0.18	0.68	0.73	0.25	1.36	0.50	0.85	0.65	0.37	0.58	0.39	0.48	0.29	0.47	0.35	0.25	0.52
χ^2	ns	ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***[d]	***

[a]Excluding abnormal vertebrae.
[b]The second figure where shown had a frequency of greater than 35%.
[c]ns: no significant difference; ***: significant difference at $P = 0.001$ level.
[d]No significant difference was obtained by excluding specimens from deep water.

consistently had a high frequency of 30 vertebrae. The significant variation with depth in Div. 2H was due to the high percentage of samples with 31 vertebrae from the shallow waters. In the other areas (Div. 2J, Subarea 3 and 4), χ^2-statistics showed significant differences in the vertebral frequencies with depth. In Div. 4X, excluding specimens from deep water (>600 m) reduced the χ^2 to a nonsignificant level. In general, vertebral number increased with depth except in Baffin Bay and Labrador waters.

The geographic variation of vertebral frequencies could be appraised from area to area by examining the frequency distributions (Fig. 2). In Baffin Bay (Subarea 0, 1) and northern Labrador (Div. 2G, and 2H), vertebral counts of 30 were dominant at all depths, and so *S. mentella* was determined to be the predominant species. However, 35.2% of the redfish collected from the 200–299 m in 2J and 41% of the redfish collected from <300 m in 3K had 29 vertebrae (Fig. 2A), indicating the presence of *S. fasciatus* at these depths. A mixture of redfishes was observed on Flemish Cap (Div. 3M): the two species intermingled in shallow waters (<300 m) but *S. mentella* dominated in deep waters (Fig. 2B). On Grand Bank (Div. 3LNO), *S. fasciatus* was predominant in shallow waters and *S. mentella* was predominant in deep waters. The two species probably mixed at a depth of 400–499 m in Div. 3L and 3N, and in deeper waters (>500 m) in Div. 30 (Fig. 2B). On St. Pierre Bank (Div. 3P), *S. fasciatus* was predominant in very shallow water (<200 m) whereas *S. mentella* was predominant in deep waters. They mixed at a wide range of depth zones (Fig. 2B). In the Gulf of St. Lawrence (Div. 4RST), *S. fasciatus* was found to dominate only in water shallower than 200 m; *S. mentella* dominated at deeper than 200 m (Fig. 2B and C). On the Nova Scotian Shelf (Div. 4VWX), *S. fasciatus* was the predominant species. *Sebastes mentella* might be present in higher proportions in waters deeper than 500 m in these areas (Fig. 2C).

The statistics for vertebrae in each NAFO Division are listed in Table 3. These were calculated by excluding all the abnormal vertebrae (fused vertebrae). A clear geographic cline of vertebrae of beaked redfishes, from a high mean value in the north to a gradual reduction toward the south, was noted, exceptions being Flemish Cap (Div. 3M) and the Gulf of St. Lawrence (Div. 4ST) (Fig. 3). No particular characteristics of abnormal vertebrae rate among areas were noted, except a peculiarly high rate (1.36%) in Div. 3K. The overall percentage of abnormal vertebrae for all areas was 0.52%.

ANAL FIN RAYS

The anal fin ray statistics for each NAFO Division are listed in Table 4. The χ^2-test of the independence of anal fin ray frequencies with depth in each NAFO Division is listed to show the homogeneity of beaked redfishes. Only the specimens from Baffin Bay (Subarea 0 and 1) and northern Labrador (Div. 2G) showed the same anal fin ray frequencies throughout all depths. Strong depth variation was indicated in all other areas. However, in Div. 2H, 4S, and 4T, no significant differences of anal fin ray frequencies were found by excluding specimens from shallow waters.

In Baffin Bay (Subarea 0 and 1) and Labrador (Div. 2G, 2H, and 2J) (Fig. 4A), the anal fin rays of 8 and 9 were both

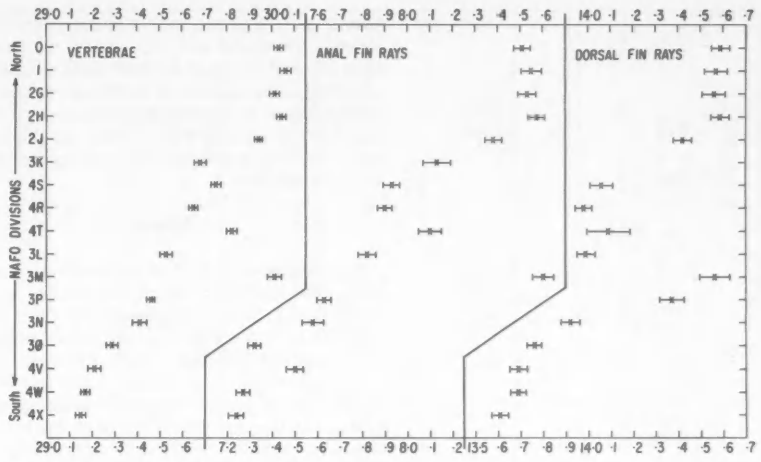

FIG. 3. Geographic clines of meristics in beaked redfishes. NAFO Divisions are in the sequence of north down to south. Horizontal bars are mean values of meristics with 95% confidence limits.

equally observed. Therefore, *S. mentella* was the predominant species. In Div. 2J, 22.8% of the specimens collected from the 200- to 299-m-depth zone had anal fin ray counts of 7, indicating the presence of *S. fasciatus*. Although the presence of *S. fasciatus* was also noted in the shallow waters of Div. 3K, *S. mentella* dominated. *Sebastes fasciatus* dominated the shallow waters of Div. 3L whereas *S. mentella* dominated the deep waters of Div. 3K and 3L. On Flemish Cap (Div. 3M), the two species intermingled in shallow waters (<400 m) and *S. mentella*, with an anal fin ray count of 9, dominated in deeper waters (Fig. 4B). *Sebastes fasciatus* was predominant in shallow waters of Div. 3N and probably mixed with *S. mentella* at a depth of around 400–499 m. In Div. 3O, *S. fasciatus* was again predominant down to 500 m and a mixture occurred in deeper water. On St. Pierre Bank (Div. 3P), *S. fasciatus* was predominant in shallow water whereas *S. mentella* was predominant in deep water. They mixed at the depth zones deeper than 300 m (Fig. 4B). In the Gulf of St. Lawrence (Div. 4RST), *S. fasciatus* dominated only in water shallower than 200 m; *S. mentella*, with 8 anal fin rays, was dominant in waters deeper than 200 m (Fig. 4B and C). In Div. 4Vn, *S. mentella* was still the dominant species. On the Nova Scotian Shelf (Div. 4Vs, 4W, and 4X), *S. fasciatus* was the dominant species. *Sebastes mentella* may be present in a larger proportion in waters deeper than 500 m in Div. 4W and 4X. A geographic cline, a reduction in anal rays from north to south, was also observed except the Flemish Cap, Banguereau (Div. 4V), and Div. 4T (Fig. 3).

Anal fin ray frequencies were then examined using cluster analysis to display the relatedness of depth and geographic components. A dendrogram (Fig. 5) describes the sequence in which clusters were formed in the amalgamation process. Six groups were observed. (1) The deepwater redfish in northeastern Newfoundland had an anal fin ray pattern of 8–9 similar to those of Baffin Bay and Labrador waters. These were determined as *S. mentella* — the Labrador and Baffin Bay group. (2) On the Flemish Cap, *S. mentella*, having an anal fin ray count of 9, was dominant. (3) *Sebastes mentella*, with 8 anal fin rays, was dominant in the Gulf of St. Lawrence. (4) A mixture of anal fin ray 7, 8, and 9 indicated the combination of *S. mentella* and *S. fasciatus*. (5) In the shallow waters of Grand Bank and Nova Scotian Shelf, *S. fasciatus* dominated with 7 anal fin rays. (6) A small portion of 8 or 9 anal fin rays suggested that *S. fasciatus* was dominant with occasional occurrence of *S. mentella*.

In summary, significant depth and geographic variation of anal fin ray frequencies were observed in beaked redfishes. It again indicated that *S. fasciatus* were distributed in shallower waters and southern areas, whereas *S. mentella* were dominant in deeper water and northern areas.

DORSAL FIN RAYS

The dorsal fin ray percentage frequency histograms with sample sizes and mean values were calculated (Fig. 6). Depth and geographic variation were again noted. Beaked redfishes in deep and northern waters had a greater number of dorsal fin rays than those of shallow and southern waters (Table 5 and Fig. 6). The patterns changed from 15–14 in Baffin Bay and Labrador waters, to 14 in deep waters of Grand Bank and in Gulf of St. Lawrence, and then to 14–13 in very shallow waters of Grand Bank and on the Nova Scotian Shelf. Exceptions for the geographic cline were Flemish Cap and the St. Pierre Bank (Fig. 3).

VERTEBRAE, ANAL FIN RAYS, AND DORSAL FIN RAYS COMBINED

As significant depth and geographic variation were observed in each meristic frequency, cluster analysis was then conducted by considering all three meristic frequencies to display better the relatedness of depth and geographic components. Cases with sample size less than 20 were excluded from the calculations. As shown by distance matrix in shaded

TABLE 4. Anal fin ray statistics of beaked redfishes for each NAFO Division. χ^2-test indicates the independence of anal fin ray frequencies with depth zones used in Fig. 4.

Statistics	Baffin Bay		Labrador Shelf			NE Nfld.	Flemish Cap	Grand Bank			St. Pierre Bank	Gulf of St. Lawrence			Nova Scotian Shelf			
	0	1	2G	2H	2J	3K	3M	3L	3N	3O	3P	4R	4S	4T	4V	4W	4X	Total
N	771	591	825	1282	1627	437	1340	1850	1279	1993	2452	2205	1588	522	1239	1608	1013	22 622
$\bar{x}$	8.51	8.55	8.53	8.57	8.38	8.13	8.60	7.82	7.58	7.32	7.63	7.90	7.93	8.10	7.50	7.27	7.24	7.88
Mode[a]	9,8	9,8	9,8	9,8	8,9	8	9	7,8	7	7	7,8	8	8	8	7	7	7	8,7
Range	7–10	7–11	6–10	7–12	6–10	5–10	6–12	6–11	6–11	6–11	6–11	6–10	6–10	7–10	6–10	6–11	5–9	5–12
$s_{\bar{x}}$	0.0195	0.0237	0.0192	0.0172	0.0172	0.0306	0.0236	0.0196	0.0226	0.0142	0.0153	0.0153	0.0171	0.0252	0.0189	0.0149	0.0153	0.0055
χ^2[b]	ns	ns	ns	***	***	***	***	***	***	***	***	***	***[c]	***[c]	***	***	***	***

[a]The second figure where shown had a frequency of greater than 35%.
[b]ns: no significant difference; **: significant difference at $P = 0.01$ level; ***: significant difference at $P = 0.001$ level.
[c]No significant difference was obtained by excluding specimens from shallow water.

form (Fig. 7), a similar pattern to the results obtained from vertebral frequencies and from anal fin ray frequencies was again obtained to support the distribution of beaked redfishes: *S. mentella* were shown to be distributed in northern and deep waters whereas *S. fasciatus* were shown to be distributed in southern and shallow waters. Even the mixing zones for beaked redfishes resembled those derived from anal fin rays and from vertebrae.

Discussion

The distributions of beaked redfishes were proposed on the basis of the assumption that *S. mentella* has higher meristic counts than *S. fasciatus*. Although Ni (1981a, b) indicated that 98% of *S. fasciatus* had anal fin ray counts of 7, and 68.7% had vertebral counts of 29, whereas *S. mentella* had 100% anal fin ray counts of 8 or greater and 99% vertebrae counts of 30 or greater, the within-species geographic variation of anal fin rays and vertebrae of the two beaked redfishes should be carefully considered to confirm their distribution. However, the general pattern of 7 anal fin rays and 29 vertebrae for *S. fasciatus* and 8 or 9 anal fin rays and 30 vertebrae for *S. mentella* has been observed throughout most of the Northwest Atlantic (Table 1). It is, therefore, reasonable to assume that the intraspecific variability is not likely to exceed the interspecies differences.

That the significantly different patterns of vertebral and anal fin ray frequencies are differences between species rather than merely geographic variation of the same species can be substantiated by the following evidence. (a) In the temporal variation study, although 47% of the 68 division-depth blocks of vertebral frequencies showed significant differences yearly, only 19.1% showed significant differences after eliminating 1 or 2 year's data (Table 2). This supported the hypothesis that different types of vertebral patterns existed for beaked redfishes. It also suggested a certain degree of vertical migration. The inference was drawn because no significant differences were found from the tests of Div. 4R (7-yr data), Div. 4S (4-yr data), and Div. 4T (6-yr data) by eliminating 1 year's data for both the <200-m and the 300- to 399-m-depth zones. (b) From the study of depth variation, I obtained no significant differences by eliminating deep- or shallow-water data (Tables 3 and 4). This also indicated that mixtures of different types of vertebral and anal fin ray frequencies occurred. (c) Two clear clusters were obtained from vertebral frequencies, from anal fin ray frequencies, and from the three meristic frequencies combined (Fig. 7) to indicate the distribution of beaked redfishes. Even the overlapping of the two clusters, indicating the zones where the two species mixed, are similar. It was understandable that no species-specific pattern could be drawn from dorsal fin ray frequencies, as it was not suggested in the discriminant function (Ni 1981b).

Significant temporal and depth variation of vertebrae and anal fin ray frequencies showed strong division-depth characteristics except for the waters between Greenland and Baffin Island. The geographic cline for vertebrae, observed by Templeman and Pitt (1961), was verified. Vertebral averages decline from 30's in Baffin Bay and Labrador waters southward either along Northeastern Newfoundland waters and

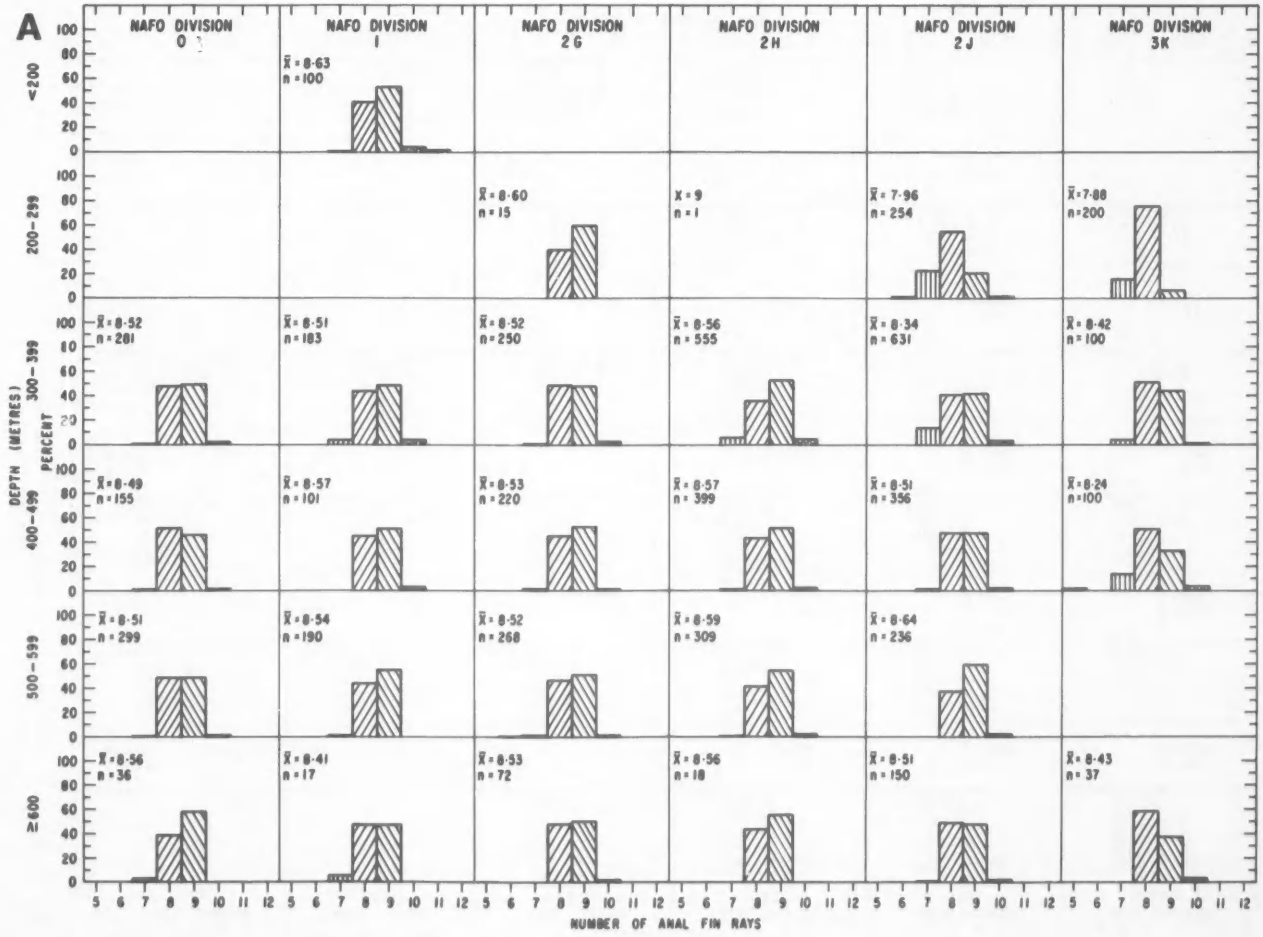

FIG. 4. Percentage frequency histograms of anal fin rays in NAFO division-depth blocks.

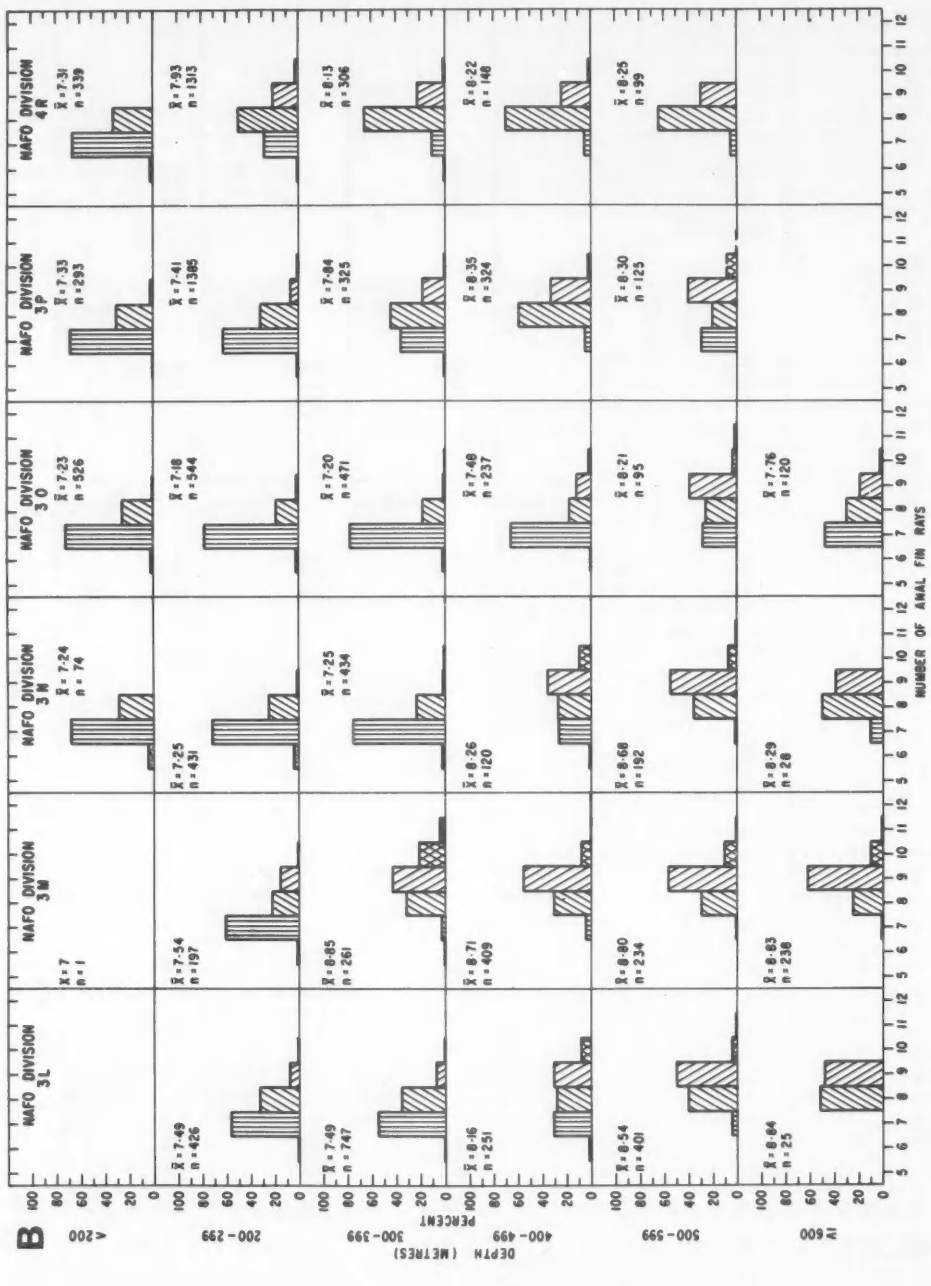

FIG. 4. *(Continued)*

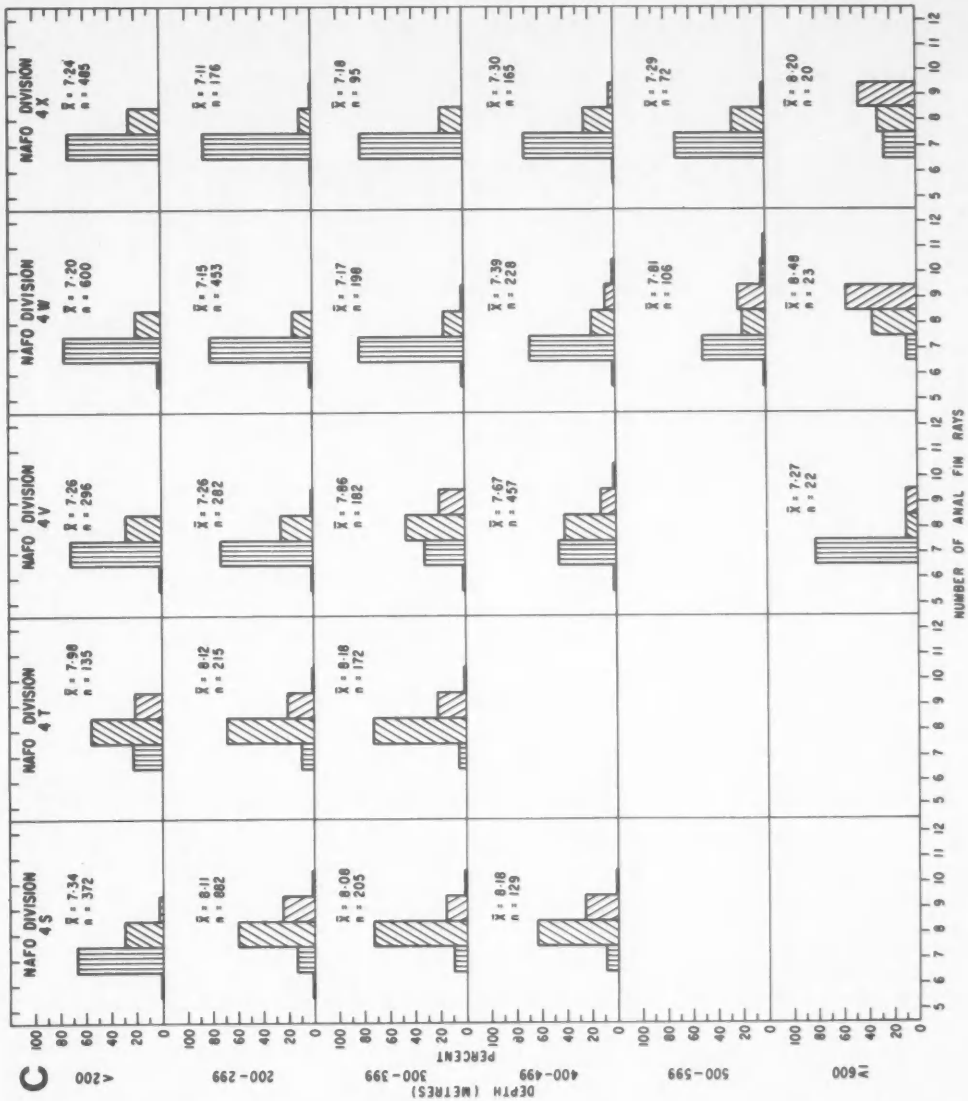

FIG. 4. *(Concluded)*

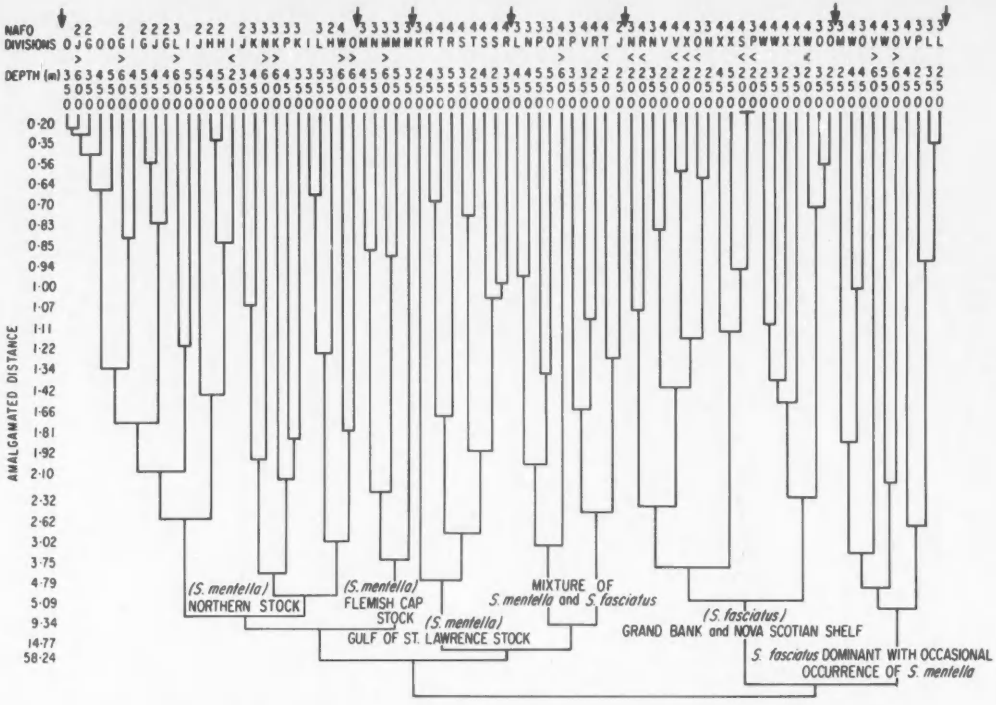

FIG. 5. Dendrographic relationship of anal fin ray frequencies among division-depth blocks. Case labels are NAFO division and depth intervals. Depth at 250 represent 200–299 m, same apply to 350, 450, and 550.

Grand Bank or along the Gulf of St. Lawrence to low 29's on the Nova Scotian Shelf. Geographic clines for anal fin rays and dorsal fin rays were also observed, there being a reduced number of rays from north to south. Exceptional area was Flemish Cap (Div. 3M) (Fig. 3).

It was interesting to note that additional meristic patterns could be ascertained especially from anal fin rays (Fig. 5). For management purposes, five hypothetical stocks in Northwest Atlantic are proposed based on these meristic taxa (Table 6). They are the Baffin Bay and Labrador stock, Flemish Cap stock, Gulf of St. Lawrence stock, Grand Bank stock, and Nova Scotian Shelf stock. Although there were no significant differences of vertebral and anal fin ray frequencies for *S. fasciatus* between Grand Bank and the Nova Scotian Shelf, the deep Laurentian Channel (>400 m) might possibly act as a barrier and two separate fishery jurisdictional stocks could be considered.

Sebastes mentella were predominant in the waters between Baffin Island and Greenland, in Labrador waters, in deep waters of the Gulf of St. Lawrence (>200 m), and of NAFO Subarea 3 (deeper than around 350–450 m), and in very deep waters of the Nova Scotian Shelf (>600 m). *Sebastes fasciatus* were distributed on the Nova Scotian Banks (<500 m), in shallow waters of NAFO Subarea 3 (<350–450 m), and in the very shallow waters of Gulf of St. Lawrence (<200 m). Therefore, *S. fasciatus* should not be called the "Labrador redfish" as in the fourth edition of "A List of Common and Scientific Names of Fishes from the United States and Canada" (American Fisheries Society, 1980). I would support Barsukov (1972), W. N. Eschmeyer (Department of Ichthyology, California Academy of Science, San Francisco, CA, personal communication), and E. J. Sandeman (Fisheries Research Branch, Fisheries and Oceans Canada, P.O. Box 5667, St. John's, Nfld. A1C 5X1, personal communication), who suggested that the common name "rosefish" be retained for *S. fasciatus* as first suggested by Taning (1949). "Bank redfish" as proposed by C. R. Robins (Division of Biology and Living Resources, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL, personal communication), and "shelf redfish," as proposed by T. J. Kenchington (Department of Biology, Dalhousie University, Halifax, NS, personal communication), are other suitable names by implying shallow water. It is appropriate to use "deepwater redfish" for *S. mentella* as it is predominant in most of the deep waters of Northwest Atlantic although it can also be found in the shallow waters of Baffin Island, West Greenland, and Labrador.

In conclusion, significant depth variation and geographic cline of meristics were observed in beaked redfishes. *Sebastes mentella*, having 30 vertebrae and 8 or 9 anal fin rays,

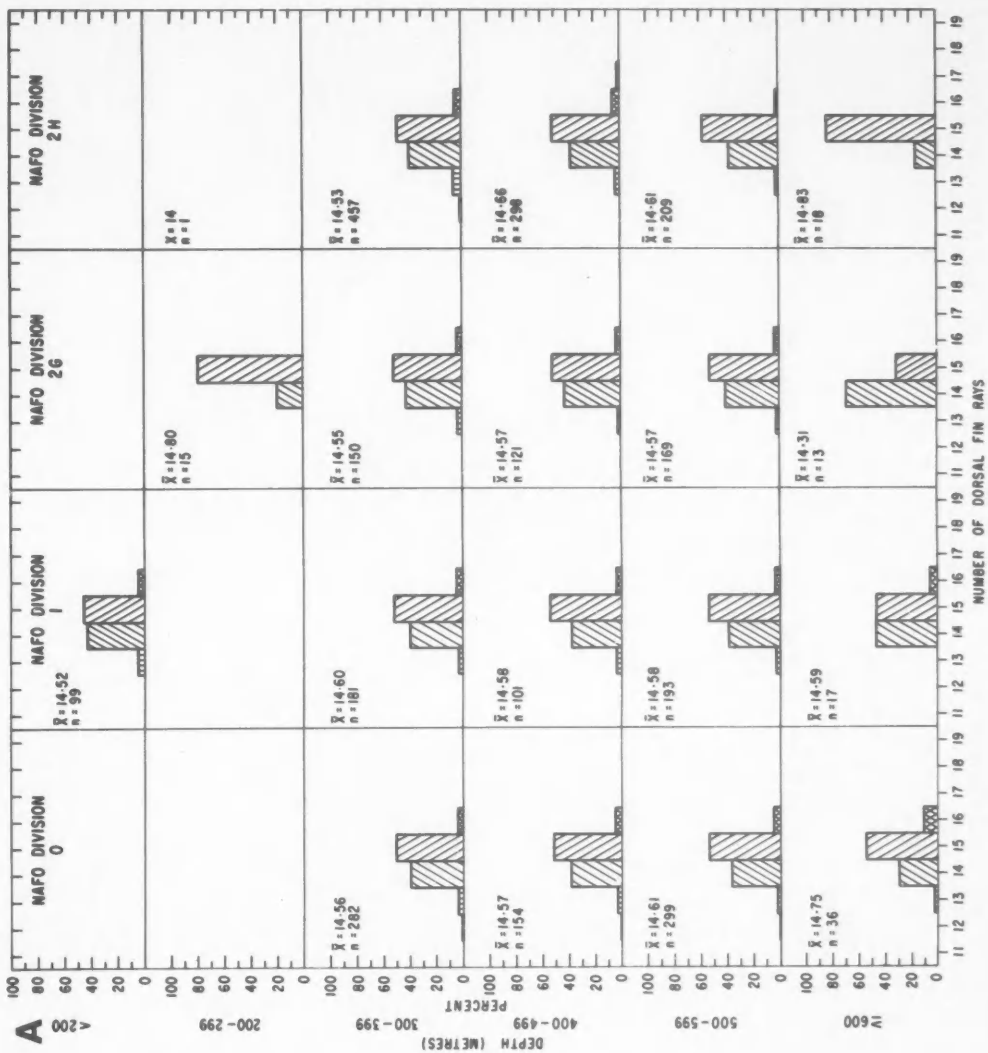

FIG. 6. Percentage frequency histograms of dorsal fin rays in NAFO division-depth blocks.

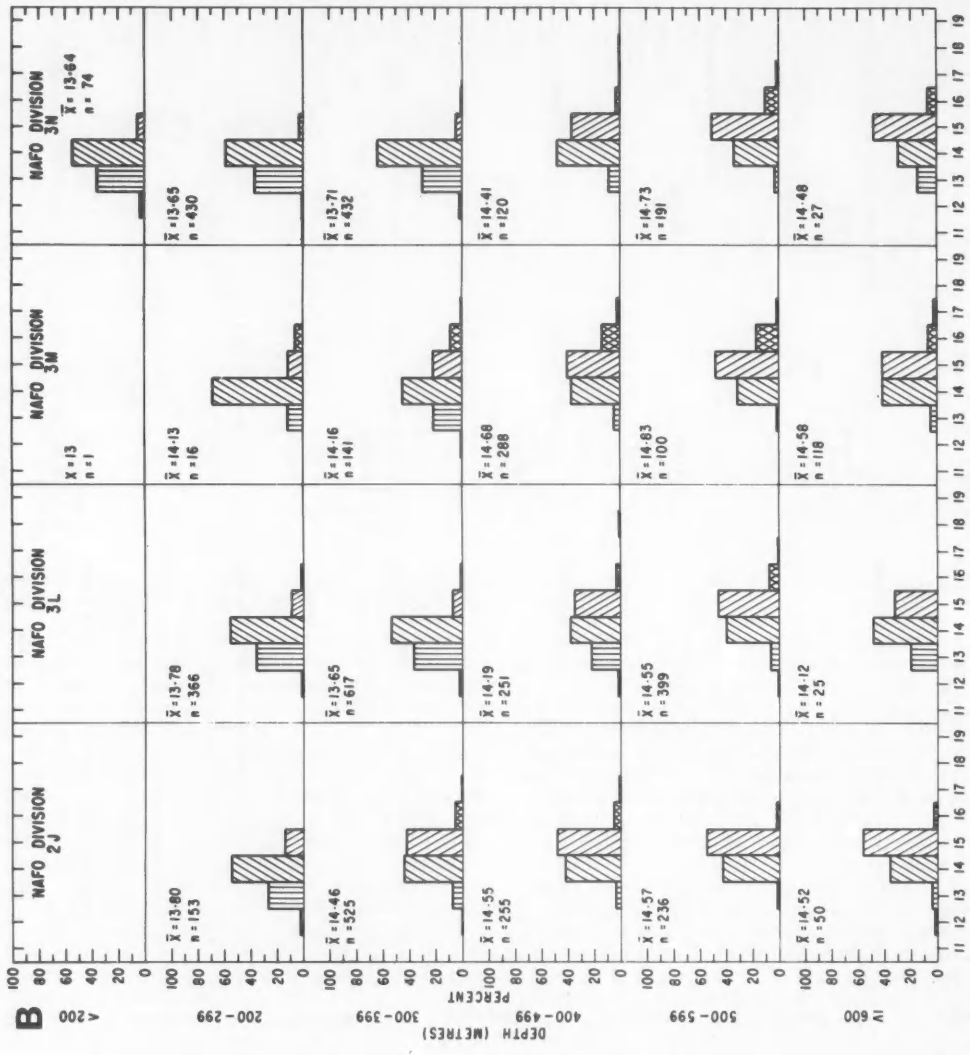

FIG. 6. *(Continued)*

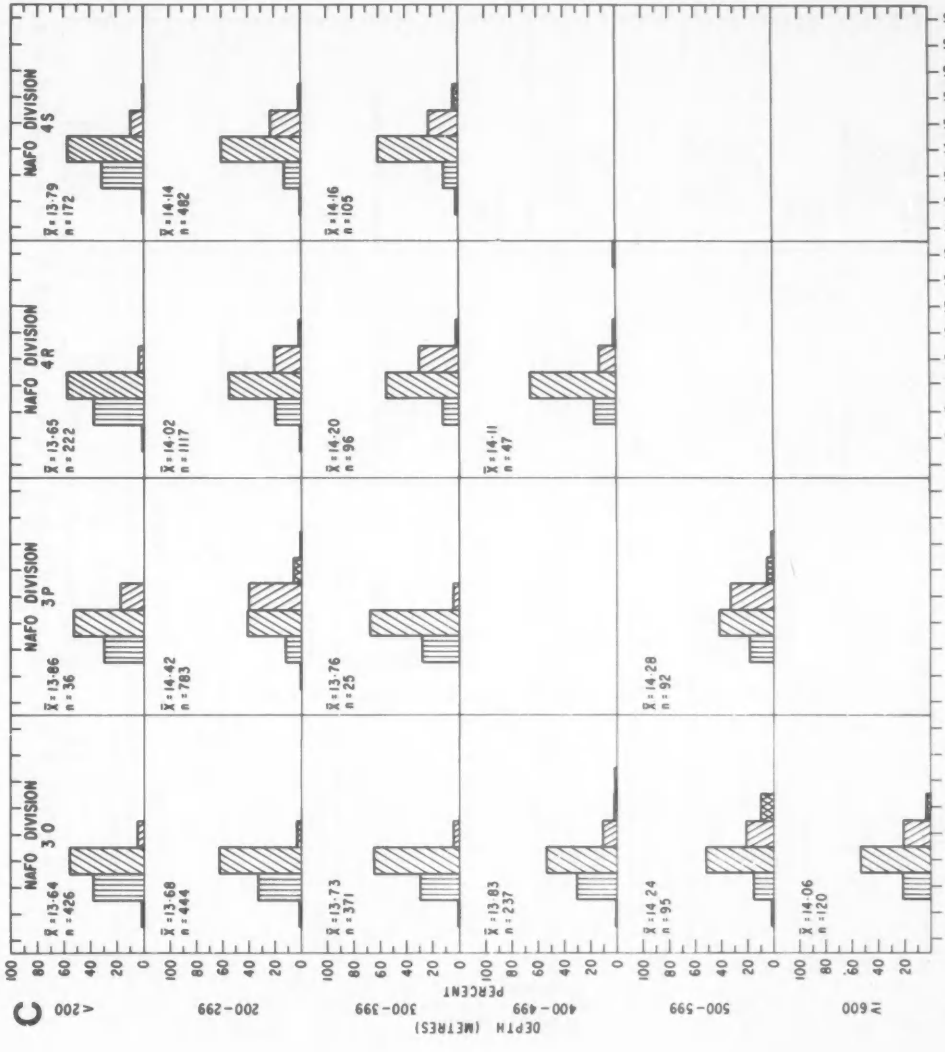

FIG. 6. *(Continued)*

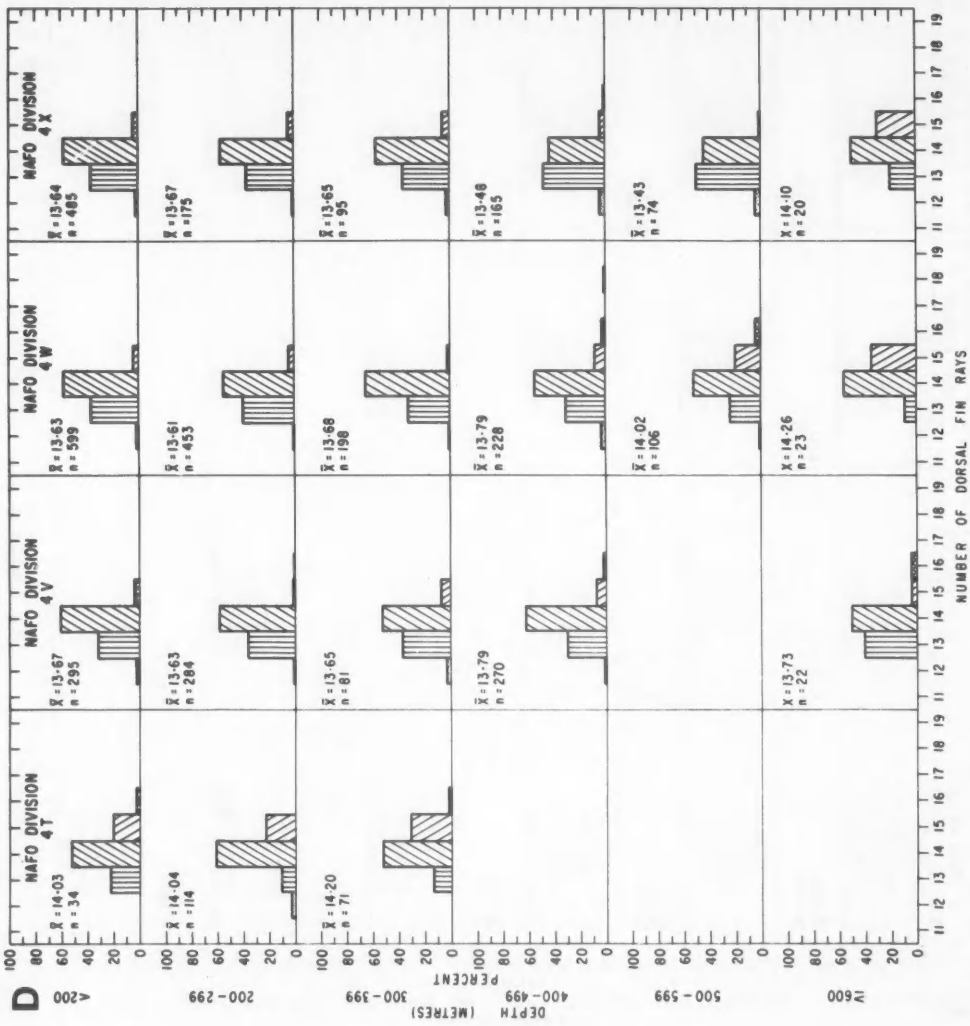

FIG. 6. *(Concluded)*

TABLE 5. Dorsal fin ray statistics of beaked redfishes for each NAFO Division. χ^2-test indicates the independence of dorsal fin ray frequencies with depth zones used in FIG. 6.

	Baffin Bay		Labrador Shelf			Flemish Cap	Grand Bank			St. Pierre Bank	Gulf of St. Lawrence			Nova Scotian Shelf			
Statistics	0	1	2G	2H	2J	3M	3L	3N	3O	3P	4R	4S	4T	4V	4W	4X	Total
N	771	591	468	983	1219	664	1658	1274	1693	936	1482	759	219	952	1607	1014	16 290
$\bar{x}$	14.59	14.57	14.56	14.58	14.42	14.56	13.99	13.92	13.76	14.37	13.98	14.06	14.09	13.69	13.69	13.61	14.07
Mode[a]	15,14	15,14	15,14	15,14	14,15	14,15	14	14	14	14,15	14	14	14	14	14,13	14,13	14
Range	12–16	13–16	13–16	12–17	12–17	12–17	12–18	11–18	11–17	12–17	12–19	12–16	12–16	12–16	11–18	12–16	11–19
$SE_{\bar{x}}$	0.0239	0.0259	0.0273	0.0213	0.0208	0.0340	0.0198	0.0213	0.0157	0.0268	0.0186	0.0250	0.0476	0.0195	0.0162	0.0192	0.0063
χ^{2b}	ns	ns	ns	*	***	***	***	***	***	***	***	***	ns	*	***	***	***

[a]The second figure where shown had a frequency of greater than 35%.
[b]ns: no significant difference; *: significant difference at $P = 0.05$ level; ***: significant difference at $P = 0.001$ level.

TABLE 6. Hypothetical stocks of beaked redfishes in Northwest Atlantic. Abbreviations are NAFO Divisions.

	Hypothetical stocks	Species	Vertebrae	Dorsal fin ray	Anal fin ray	Note
1.	Baffin Bay (Subarea 0 and 1) Labrador Coast (2GHJ) Northeastern Newfoundland waters (>300 m) (3K)	*S. mentella*	30	14,15	8,9	<300 m of 2J and 3K may have *S. fasciatus*
2.	Flemish Cap (3M) (≥300–400 m)	*S. mentella*	30	15	9	<300 m are *S. fasciatus* and *S. marinus*
3.	Gulf of St. Lawrence (4RST)	*S. mentella*	30	14	8	<200 m are *S. fasciatus*
4.	Grand Bank (3LNO)	*S. fasciatus*	29	14	7	3LN: >500 m are *S. mentella*
5.	Nova Scotian Shelf (4VWX)	*S. fasciatus*	29	14,13	7	>600 m may have *S. mentella*

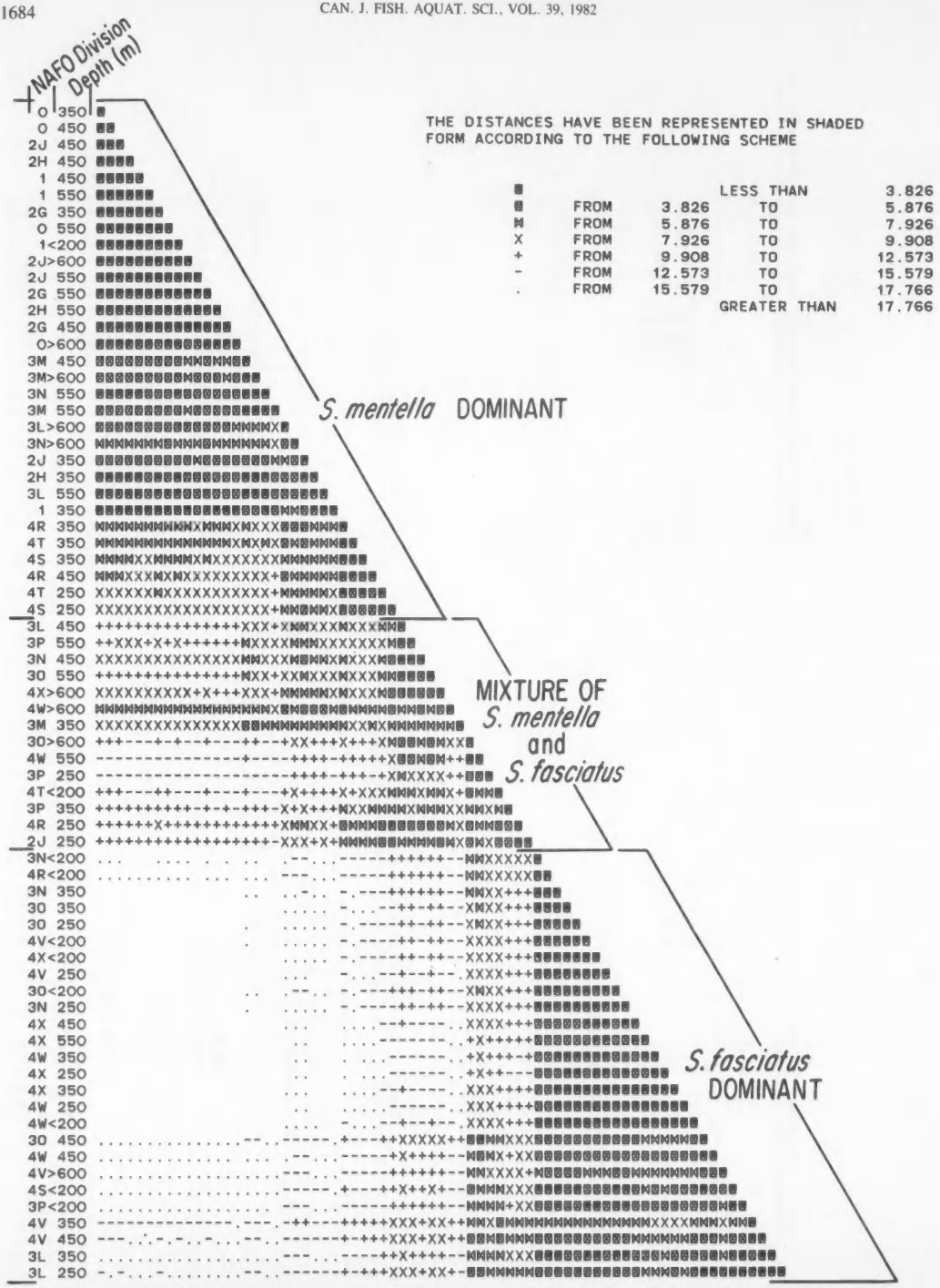

FIG. 7. Cluster analysis of vertebrae, anal fin ray, and dorsal fin ray frequencies. Case labels are NAFO division and depth intervals. Depth at 250 represent 200–299 m, same apply to 350, 450, and 550.

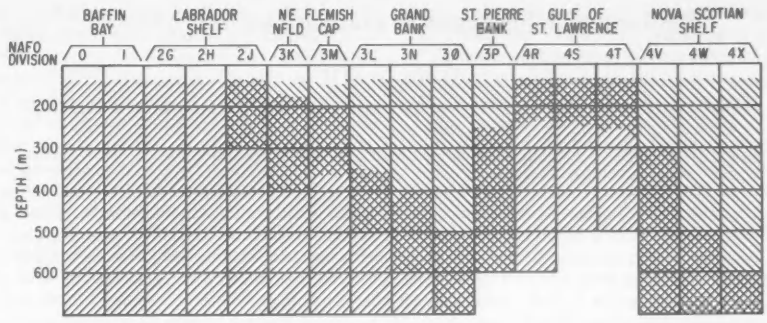

FIG. 8. The distribution of *S. mentella* and *S. fasciatus* in division-depth blocks. ▩: *S. mentella*, ▩: *S. fasciatus*, ▩: mixture of *S. mentella* and *S. fasciatus*.

dominated in northern and deep waters whereas *S. fasciatus*, having 29 vertebrae and 7 anal fin rays, distributed in southern and shallow waters. A distribution map for beaked redfishes in Northwest Atlantic is proposed (Fig. 8).

Acknowledgments

I thank Dr W. Templeman and Mr E. J. Sandeman for the privilege of using previously collected data; Mrs B. M. Bruce, Mr E. M. LeGrow, and Redfish Section staff, who helped collect, compile, and tabulate the data; Dr C. C. Lee of Memorial University of Newfoundland for his suggestions on statistics; and Mrs C. A. Gavaris, Mr D. B. Atkinson, Drs W. N. Eschmeyer, D. J. Fairbairn, and G. R. Lilly for their valuable comments.

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Reproductive Strategies in Flatfish: A First Synthesis

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ROFF, D. A. 1982. Reproductive strategies in flatfish: a first synthesis. Can. J. Fish. Aquat. Sci. 39: 1686 – 1698.

This paper examines the factors determining the age and size at maturity of temperate species of flatfish. In some species maturity is governed by size, not age. I propose that the most likely determinant of this size is size-dependent predation. Many species fast during the winter months and rely upon reserves stored in the body tissues to provide energy for gonad maturation. The stress placed upon a fish by the breakdown of body tissues may be very substantial and could significantly affect the predation function. Under these conditions selection may favor the deferment of maturity until a size is achieved at which predation is relatively low. I suggest that such a mechanism may account for the extreme geographic variation in life history parameters of American plaice, *Hippoglossoides platessoides*, and witch flounder, *Glyptocephalus cynoglossus*. The generality of winter cessation of feeding suggests that the above hypothesis may also be applicable to a wider range of species. By contrast, in several species, age has been shown to be more important than size in determining the onset of maturity. I suggest that age is likely to be more important than size in early maturing species; in these a decrease in the age at maturity may be, temporarily at least, prevented by genetic/physiological constraints imposed by the time required for oocyte development . This hypothesis is tested and supported by historical data on several European species. Finally, I examine the intra- and inter-specific patterns of allocation to fecundity. Interspecific differences in allocation to reproduction are greatly reduced but not eliminated when egg volume is taken into consideration. Factors influencing the observed allocation patterns are examined.

Key words: pleuronectiformes, life history, reproduction, fecundity, maturity

ROFF, D. A. 1982. Reproductive strategies in flatfish: a first synthesis. Can. J. Fish. Aquat. Sci. 39: 1686 – 1698.

L'article qui suit contient une analyse des facteurs influant sur l'âge et la taille de maturité d'espèces de poissons plats des mers tempérées. Chez certaines, la taille et non l'âge détermine la maturité. Selon moi, le déterminant le plus probable de cette taille est la prédation dépendante de la taille. Plusieurs espèces jeûnent pendant les mois d'hiver et dépendent, pour l'énergie nécessaire au développement des gonades, des réserves emmagasinées dans les tissus du corps. Le stress causé par la rupture de ces derniers peut être sévère et susceptible d'influencer la fonction de prédation. Dans ces conditions, la sélection favoriserait le retard de la maturité jusqu'à ce que soit atteinte une taille à laquelle la prédation est relativement faible. Je crois donc que l'extrême variation géographique des paramètres du cycle vital de la plie canadienne, *Hippoglossoides platessoides*, et de la plie grise, *Glyptocephalus cynoglossus*, peut s'expliquer par un tel phénomène. Le fait que l'arrêt de l'alimentation en hiver soit général donne à penser que l'hypothèse s'appliquerait à une grande variété d'espèces. Par contraste, chez plusieurs espèces, il a été démontré que l'âge est plus important que la taille comme facteur réglant le début de la maturité. À mon avis, l'âge est probablement plus important que la taille chez les espèces à maturité hâtive; chez ces dernières, un abaissement de l'âge de la maturité peut être, du moins temporairement, empêchée par les contraintes génétiques/physiologiques du développement des ovocytes. Cette hypothèse a été testée, et des données historiques sur plusieurs espèces européennes l'appuient. Finalement, j'examine les modalités intra et interspécifiques de l'allocation des réserves à la fécondité. Si l'on tient compte du volume des oeufs, les différences interspécifiques de cette allocation sont considérablement réduites. Les facteurs influant sur les modalités de la répartition sont examinés.

Received February 24, 1982
Accepted August 23, 1982

Reçu le 24 février 1982
Accepté le 23 août 1982

Printed in Canada (J6826)
Imprimé au Canada (J6826)

REPRODUCTION is not a free enterprise. Energy channeled into reproductive tissues may detract from the growth potential of an organism or its survival. For fish in general a 'growth cost' of reproduction may be inferred from the frequent observation that the rate of growth decreases with the onset of maturity. The 'survival cost' of reproduction cannot be so easily demonstrated. It has, however, been suggested as being an important component in shaping the life history characteristics of such diverse organisms as rotifers and cladocera (Snell and King 1977; Lynch 1980), lizards (Tinkle 1969; Shine 1980), salmon (Schaffer and Elson 1975), and deer mice (Fairbairn 1977). One of the most important traits that is moulded by the above two costs to reproduction is the age at maturity (G. C. Williams 1966; Gadgil and Bossert 1970; Schaffer 1974a, b; Smith and Fretwell 1974; Bell 1976, 1980).

In general within poikilothermic species, fecundity increases with body size and hence optimizing the age at first reproduction means trading off future increased egg production associated with growth (assuming that energy available for growth and reproduction is limiting) against the advantages of early maturity (Roff 1980a, b; 1981a, b). In many fishes maturity appears to be more closely related to body size than age (Alm 1959; Pitt 1975). Such a relationship may arise because of the interaction between fecundity and body size mentioned above. Another reason may be that the probability of surviving the attempt to reproduce or subsequent survival may be size dependent. In the first case fecundity is reduced whereas in the second the animal may not even survive to reproduce; it is therefore most likely that size-dependent survival underlies the size dependence of maturity rather than the size dependence of fecundity. In many temperate marine fishes, feeding is decreased or even ceases during the winter. During this winter fasting period, the gonads are matured utilizing reserves stored in the body or using the body tissues themselves (Love 1970). This effort causes the fish to become physically weakened and hence more susceptible to predation, disease, or environmental stress. Depending upon the relative size of predator and prey there may be a threshold size beyond which the prey are relatively invulnerable to predation. Under these circumstances reproduction may be deferred until this threshold size is achieved. In this paper I present evidence in support of this hypothesis with respect to American plaice, *Hippoglossoides platessoides*, and other temperate species of flatfish.

Studies by deVeen (1970, 1976) suggest that in North Sea sole, *Solea solea*, age, not length, might be the most important determinant of maturity. Whereas a change in size at maturity can be continuous, a change in the age at maturity must, because of the seasonal nature of breeding, occur in unit steps. If the optimum size is large, we may expect a large variation in the age at first maturity. The reasons for this are twofold: firstly, differences in growth between individuals will lead to variation in the age at which the requisite size is reached. Fish breeding early are likely to show less variation in the age at first maturity because there is less time for differences in growth rate to produce significant divergence in size. The second reason lies in the relationship between the rate of increase, r, and generation length. The rate of increase is approximately inversely proportional to the mean generation length (Andrewartha and Birch 1953, p. 41). For a fish breeding late, the mean generation length will be large and the rate of increase will be little affected by 1- or 2- yr changes in the age at first reproduction. However, unit changes in the age at first reproduction can have a profound effect on the rate of increase when the mean generation length is of the order of 3 or 4 yr. Selection will therefore act very strongly on the early maturing fish while it will be relatively ineffectual on late maturing populations. A change in conditions favoring a decrease in the age at maturity will not, initially at least, produce a change in the stock because there are few or no individuals that are physiologically capable of initiating oocyte development at an earlier age. In the second part of this paper I present evidence from other species that such genetic/physiological factors can be significant constraints on at least the short-term adaptive response to changing conditions.

Reproduction requires the allocation of resources: this allocation may decrease growth or increase mortality. Thus, not only will the age at first reproduction be dictated in part by the availability of surplus energy but subsequent reproductive episodes will require the optimal allocation of energy. In the third part of this paper I examine how the allocation of energy to reproduction varies both within and between species.

Importance of Size in the Reproductive Life History

INITIAL OBSERVATIONS

One of the most remarkable features of *H. platessoides* is the difference between the reproductive life history of the species in Newfoundland waters compared to those around Scotland. In the former environment female American plaice mature at about 14 yr of age and live to be as old as 24 yr (Pitt 1966, 1967; Minet 1972) whereas in Scottish waters maturity is attained at age 3 and the maximum life span is only 6 yr (Bagenal 1955a, b, 1957). I know of no other vertebrate species that shows such a wide variation in its life history. There must clearly be a substantial selective pressure on the Newfoundland stocks to cause them to delay reproduction for so long. There are two reasons to believe that in these stocks maturity is more closely related to size than age. Firstly, in both males and females the age at maturity varies quite considerably between stocks but the sizes at maturity are quite similar (Table 1). For example, the Flemish cap stock matures at age 7.76 yr at a length of 41.3 cm whereas the population in St. Marys Bay matures at 16 yr at a length of 40.2 cm. Secondly, during the 1950s to 1970s the growth characteristics of the Grand Bank stocks showed substantial changes but while the age at maturity declined the size at maturity did not change (Pitt 1975). Why should maturity be related to size?

American plaice in the West Atantic do not feed during the winter months (Minet 1973; T. Pitt, Fisheries and Oceans, P.O. Box 5667, St. John's, Nfld., personal communication). Development of the reproductive tissues takes place at this time and depends largely on reserves stored during the previous summer (MacKinnon 1972). In flatfish, energy reserves are accumulated in the liver and muscle tissues (Love 1970). Muscle tissues may also be degraded to provide energy for

gonad development and maintenance during the winter fast. Degradation of the muscle tissues can be measured by a marked decrease in protein and a high increase in water content of the flesh. This 'jellied' condition has been found in winter flounder, *Pseudopleuronectes americanus* (Pearcy 1961), yellowfin sole, *Limanda aspera* (Kizevetter et al 1965), and witch flounder, *Glyptocephalus cynoglossus* (W. R. Bowering, Fisheries and Oceans, P.O. Box 5667, St. John's, Nfld. personal communication), but reaches a remarkable degree in American plaice: in the most 'jellied' specimen found by Templeman and Andrews (1956) the muscle consisted of 2.83% protein, 0.06% lipid, and 96.18% water. As noted by Love (1970), 'the ability of such a fish to swim is a source of wonder.'

In all of the above species the jellied condition is present only in mature animals indicating that it is the mobilization of resources for gonad development, rather than simply maintenance, that is the reason for the degradation. Not all muscle tissue is degraded during starvation. In experiments with plaice and cod it has been found that the white myotomal muscles are selectively utilized (Walker 1971; Johnson and Goldspink 1973a). These muscles are used for bursts of activity and high-speed cruising (Bone 1966; Roberts 1969; Pritchard et al 1971; Johnston and Goldspink 1973a, b, c). Their reduction during starvation or gonad maturation may inhibit the ability of a fish to avoid capture by a predator or to capture active prey itself.

The stress upon a fish during starvation undoubtably increases its susceptibility to disease, adverse environmental conditions, and predation. The deleterious effects of parasites on the health of a fish and upon both its immediate and long-term survival chances are well documented for both marine and freshwater species (Petrushevski and Shulman 1958; Sinderman 1966; H. H. Williams 1966; Chubb 1973). Heavy infections of parasites may cause sluggishness, impaired swimming ability, emaciation, increased respiration, and death (Mann 1952; Kabata 1958; Millemann and Knapp 1970; Lester 1971; Lester and Adams 1974; Pennycuick 1971; Van Den Broek 1979). Mortality from parasite infection may increase with starvation (Walkley and Meakins 1970) or spawning (Delisle 1972). A high infection of parasites, though it may not be the primary cause of death, increases the likelihood of dying from other causes and this probability is almost certainly increased when the stresses of starvation and spawning are added. The mating process itself, because it brings fish into close proximity, may increase the probability of being infected with disease. In plaice, *Pleuronectes platessa*, and flounder, *Platichthys flesus*, for example, transmission of the viral disease lymphocystis appears to occur during spawning (Russell 1974). Although this disease appears not to be generally fatal (Templeman 1965), it illustrates the potential hazards associated with spawning as it is reasonable to assume that other more deleterious diseases may be transmitted at this time.

Changes in temperature place stress on the osmoregulatory mechanism and because the maintenance of this system requires energy (Love 1970), fasting may reduce a fish's ability to maintain the necessary internal milieu. The massive mortalities of flatfish, cod, and other marine species in the North Sea during the severe winter of 1962–63 may have been caused primarily by a breakdown of the osmoregulatory mechanism (Woodhead 1964a, b). Severe winters in the North Sea are not infrequent, one occurring in 1929 (Lumby and Atkinson 1929) and another in 1946 (Simpson 1953). In both of these cases many fish died. Large numbers of dead fish during and following a cold period have been reported from Norway (Dannevig 1930), Newfoundland (Templeman 1975), Maine (Bigelow and Schroeder 1953), and Florida (Storey and Gudger 1936; Miller 1940; Galloway 1941). Flatfish species reported to die in large numbers as a result of the low temperatures are soles (Lumby and Atkinson 1929; Simpson 1953; Woodhead 1964a, b), American plaice (Templeman 1975), and winter flounder (Bigelow and Schroeder 1953), though in the North Sea almost all commercial species of flatfish were found to be affected by severe winters. Many of the flatfish dying from the North Sea kills (1929, 1946, and 1962) were infected with an unidentified disease that caused skin flaking and breakdown of the muscle tissues. Woodhead (1964a, b) suggested that this disease probably caused some deaths. The high mortalities during severe winters underline the advantage of being physiologically fit during both these types of conditions and the more usual winter conditions—during the cold period a fish physically weakened by starvation may be unable to maintain its osmotic balance, resist disease, or escape predation. During the winter months death may not result directly from an inability to regulate internal body conditions but the stress that may

TABLE 1. Size and age at maturity in American plaice.[a]

Location	Minimum size at maturity, cm	Size at 50% maturity, cm	Age at 50% maturity, yr	Winter feeding
		Females		
St. Marys Bay	32	40.2	15.15	No
St. Pierre Bank	42	44.6	14.21	No
St. Pierre Bank[b]	35	44.3	14	No
NE Grand Bank	36	42.6	13.98	No
SE Grand Bank	42	46.8	12.17	No
SW Grand Bank	42	53.6	13.63	No
Flemish Cap	36	41.3	7.76	No
Magdalen shallows	34	41	10	Yes
Trondheimsfjord	19	—	—	Yes
Passamaquoddy Bay	13[c]	—	—	Yes
Scotland	12	20	3	Probably
		Males		
St. Marys Bay	16	20	5	No
St. Pierre Bank	20	27.8	7.48	No
St. Pierre Bank[b]	15	22.7	6	No
NE Grand Bank	18	24.0	7.33	No
SE Grand Bank	20	24.8	5.32	No
SW Grand Bank	20	29.3	5.46	No
Magdalen Shallows	15	25	6	No
Trondheimsfjord	17			Yes
Passamaquoddy Bay	13[c]			Yes

[a]Data sources: Bagenal 1957; Huntsman 1918; Johnsen 1944; Lande 1976; Minet 1972; Pitt, 1966, personal communication; Powles 1965.

[b]From Minet 1972.

[c]Sexes not separated.

TABLE 2. List of predators of flatfish in the West and East Atlantic. Data from Wheeler (1969) and Leim and Scott (1966).[a]

West Atlantic		East Atlantic	
Species	Size	Size (cm)	Species
Scyliorhinus stellaris	152		
Lamna nasus	300	300	*Lamna nasus*
Galeorhinus galens	150		
Squalus acanthias	122		
Squatina squatina	183		
Torpedo nobiliana	180		
Raja oxyrinchus	130		
Raja batis	190	150	*Raja laevis*
Conger conger	183		
Gadus morhua	150	150	*Gadus morhua*
Molva molva	200		
Hippoglossus hippoglossus	250	250	*Hippoglossus hippoglossus*
Scophthalmus maximus	100		
Lophius piscatorius	198	120	*Lophius americanus*

[a]Notes: Predators such as sea ravens that eat only small fish are not included. *Raja laevis* and *Lophius americanus* are ecological homologues of *Raja batis* and *Lophius piscatorius*, respectively.

come from a physically weak fish attempting to maintain its physiological balance may increase the fish's chance of falling victim to disease or predation.

A HYPOTHESIS

Because of changes in metabolism the amount of reserves required by fish in general for maintenance per unit time, measured as a percentage of body weight, decreases with size (Edwards et al 1971; MacKinnon 1973; Niimi and Beamish 1974; Jones and Hislop 1978). Thus a larger fish may be better able to tolerate the stress placed upon it by gonad maturation. This is clearly shown in the immature American plaice of the Scottish stock; during the winter months when their feeding is probably reduced, the small fish are relatively lighter than the large (fig. 2 of Bagenal 1957). However, a more likely cause of significant size-dependent mortality associated with weakness from fasting and gonad development is predation. The increased risk of predation may arise because of the weakened condition of the fish per se and/or it may be due to an increase in foraging activity after spawning when the depleted reserves of the fishes must be replenished. I hypothesize that predation on American plaice is size dependent and that at the size at which they are observed to mature they are of such dimensions that predation mortality is not significantly increased by the stress incurred during gonad maturation. Fish smaller than this size, by diverting energy from maintenance into reproduction, greatly increase their probability of being preyed upon. In Scottish waters temperatures are higher than around Newfoundland (MacKinnon 1973) and I hypothesize that American plaice can feed throughout the winter in the former waters and hence are not subject to the same stress during and after gonad maturation. An implicit assumption of this hypothesis is that the predatory pressure in the two areas is more or less the same. A list of species recorded to eat flatfish in European and American waters is given in Table 2: I have omitted such species as sea ravens that eat only small fish. There are more sharks and rays recorded as eating flatfish in European waters but otherwise the number of species is similar, either the same species occurring on both sides (porbeagle (*Lamna nasus*), Atlantic cod (*Gadus morhua*), Atlantic halibut (*Hippoglossus hippoglossus*) or pairs that are ecological homologues (e.g. the angler fishes *Lophius piscatorius* and goosefish (*Lophius americanus*)).

TESTS OF THE HYPOTHESIS

The hypothesis advanced above can be divided into two components,

(a) the size at maturity is determined primarily by the length of the winter fast.

(b) the proximal mechanism for (a) is the susceptibility of weakened fish to size-selective mortality, most likely predation.

Evidence for (a) can be obtained from analysis of other stocks.

COMPARISON WITH OTHER STOCKS

Feeding data are available on two stocks of American plaice, one in Trondheimsfjord, Norway, and the other in Passamaquoddy Bay, Canada. In both stocks feeding occurs throughout the year (Lande 1976; Tyler 1971). I therefore predict that although these stocks are widely separated, their life history characteristics should match those of the Scottish stocks. Johnsen (1944) reported the minimum size for mature females in the Trondheim region to be 19 cm with a maximum size of about 35 cm.

As predicted these lengths are closer to the Scottish population than the Newfoundland stocks. In Passamaquoddy Bay maturity is first reached at about 13 cm (Huntsman 1918, sexes not separated) at an age of 2 yr. The maximum age of these fish is 6 yr. These characteristics are very similar indeed to the Scottish stock and dramatically different from the

TABLE 3. Size and age at maturity in female witch.[a]

Location	Minimum size at maturity[b]	Size at 50% maturity	Minimum age at maturity	Age at 50% maturity	Winter feeding
St. Pierre Bank	36	44	7	10.21	No
SW Grand Bank	40	44	6	8.42	No
Hawke Channel and NE Newfoundland Shelf	36	50	7	8.62	No
Gulf of St. Lawrence	34	40	8	9.95	No
Scotland	21	—	3	—	Yes

[a]Data sources: Bagenal 1963a; Bowering 1976 personal communication; Rae 1969.
[b]All lengths in centimetres.

Newfoundland stocks to which they are much closer geographically.

The witch flounder *G. cynoglossus* lives in close proximity to the American plaice and like this species undergoes a winter fast in Newfoundland waters (Bowering personal communication). In Scottish waters, however, the witch feeds throughout the year (Rae 1969). The Newfoundland stocks mature at very close to the same length as the American plaice, though the ages at maturity are quite different (Table 3). Scottish witch mature at 3 yr of age and at the relatively small size of 21 cm, characteristics that make them most similar to the Scottish American plaice. Thus, whatever factor is determining the size at maturity in American plaice is probably determining the size at maturity in witch.

The winter flounder *P. americanus* occurs throughout the coastal waters of Atlantic Canada and in both Newfoundland waters and those of Passamaquoddy Bay it undergoes a winter fasting period (Tyler 1971; Kennedy and Steele 1971; Fletcher and King 1978). Thus one would predict that unlike the American plaice which shows very different life history characteristics in the two regions, the winter flounder stocks of these areas should be similar. Indeed their growth rates are very similar (Kennedy and Steele 1971) and the lengths at which the females mature are about the same (25 cm in Newfoundland: Kennedy and Steele 1971, 27 cm in Passamaquoddy Bay; A. V. Tyler, Department of Fisheries and Wildlife, Oregon State University, Corvalis, OR 97331, personal communication).

EVIDENCE FOR SIZE-SELECTIVE PREDATION

American plaice from the Magdalen Shallows are similar in their life history characteristics to those from Newfoundland waters. They mature at about the same size (Table 1) and live to approximately the same age (Powles 1965; Pitt 1967). Like the Newfoundland fish they do not feed during the winter (Powles 1965). American plaice in the Magdalen Shallows are heavily preyed upon by cod and from observation Powles estimates that cod feed only upon fish that are less than 35 cm in length. The minimum size at which female American plaice of the Magdalen Shallows mature is ~34 cm, a size that is close to the Newfoundland stocks. These data support the hypothesis that American plaice in these waters defer maturity until their size renders them relatively immune from predation.

Male American plaice on the Magdalen Shallows mature at a smaller size (50% mature at 25 cm) than the females, as is common in flatfish species. But males do not have to commit as much reserves to gonad production as females and hence may not reach the same weakened physical condition. In the Grand Bank stock neither males nor females show the jellied condition until they reach a length of 44–57 cm (Templeman and Andrews 1956), although the 50% maturity point for males is about 25 cm as compared to 45 cm for females (Pitt 1966). The ovary weights of females 35 cm in length from St. Margarets Bay, Nova Scotia, vary from 1.6% of body weight after spawning to about 10% prior to spawning, whereas the ovaries of immature females of this size amounted to only 0.8% of the body weight throughout the year, and the testes weight of males 25 cm in length varied from 1 to 2% of body weight during the year (MacKinnon 1972). Observations on dab (*Limanda limanda*) indicate that females must store about three times more energy in their gonads than males (Pandian 1970). Male American plaice reproducing at 25 cm have to commit only a small amount of energy to gonad production and hence the lack of the jellied condition at this size is not surprising. No data are available on the commitment of larger-sized males to testes maturation: the jellied condition of these fish may be the result of either (or both) the single commitment in a year to the gonads or the accumulated drain placed on the body tissues over a number of years.

Thus far we have considered the importance of size in determining the onset of maturity. There are, however, circumstances in which age appears to play an important role. These circumstances are examined in the next section.

Importance of Age in the Reproductive Life History

INITIAL OBSERVATIONS

Starting in 1963 there was an increase in the size at age of the North Sea sole *S. solea* (de Veen 1970, 1976), and in 1969 there was an upward shift of 2½ cm in the size at which 50% of the females mature. This shift has been maintained since then but no change has been observed in the maturity/age relationship (de Veen 1976). These observations are somewhat at variance with each other: if the length at age began to change in 1963 but there was no change in the maturity/age

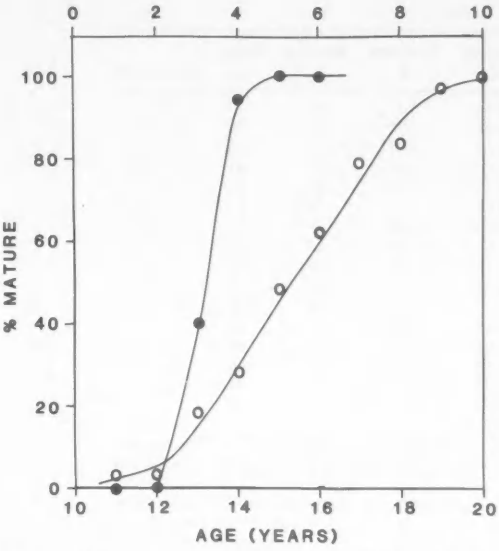

FIG. 1. Maturity at age for American plaice and North Sea sole. Data from Pitt 1964 and De Veen 1976. Bottom axis shows age of American plaice (○); top axis (●), North Sea sole.

relationship, then there must be a systematic and continuous change in the maturity/length relationship. A 1- or 2- yr delay might be expected as the change in the size at age occurred first only in age groups 1−3 but some change should have been noticed by 1966. This failure to detect any shift may be due to inadequate sampling, samples being obtained from commercial landings in which fish less than 24 cm (2-yr-olds) are poorly represented. The lack of any further change after 1968 is probably due to the very small change in the size at age since then. The change in the maturity/length relationship with the onset of heavy fishing by beam trawl appears to be more than coincidence as the data of Holt (1892), Büchman (1934), and de Veen (1976) indicate that the size at maturity remained remarkably constant during the 70 yr prior to the changes reported in the 1960s.

Why in this case might the onset of maturity be more closely related to age than size?

A HYPOTHESIS

I suggest that the reason for the contrary result observed in the sole is that genetic/physiological constraints have prevented a decline in the age at maturity of the fish. It is to be expected that each species or distinct population will have some characteristic development period for oocyte formation. In winter flounder, for example, a 3-yr period is required to mature the oocytes (Dunn and Tyler 1969; Dunn 1970). There is evidently no physiological constraint to a lower age at maturity in flatfish in general as some tropical species mature after 1 yr (Seshuppa and Bhimacher 1953, 1954). For the reasons given earlier (see Introduction) selection will act strongly on early maturing stocks for the age at maturity thereby leading to a relatively low amount of genetic variability for this trait. Thus, if selection pressures on an early maturing stock change, favoring a reduction in the age at maturity, the response may be slow because there does not exist in the population individuals that are at an earlier age. This can be seen by comparing the age capable of maturing at maturity of the sole with that of Newfoundland American plaice (Fig. 1); there is clearly considerable variation in the age at maturity of American plaice. On the other hand there is little variation in the sole, and no samples in any year showed females maturing at age 2 (de Veen 1976). Therefore, in the latter case the selection pressure for a decrease in the age at first maturity may have to be large and applied for a long period to have a significant effect. The average age at maturity for female sole is 3.2 yr and I suggest that the failure to decrease significantly its average age at maturity is due to these physiological constraints. In the face of such constraints the excess energy is channeled into growth and as a result the fish grow larger by the time they are 3 yr old.

TESTING THE HYPOTHESIS

Tests of the above hypothesis require historical data on changes in the age and length at maturity. If the hypothesis is correct fish that mature at a relatively late age will show shifts in their age at maturity, whereas fish that mature early will show shifts in their size at maturity, at least initially. One might also expect that in species maturing early, maturity will be correlated more with age than size.

The American plaice of the Newfoundland region mature late (12 yr) (Pitt 1975) and as already described there have been changes in the age at maturity but not size. However, the Scottish stocks mature at a much earlier age (3 yr) and, as predicted, maturity in these is more closely correlated with age than size (Bagenal 1957).

Further evidence in support of the genetic/physiological hypothesis is given by changes in the reproductive parameters of plaice, *Pleuronectes platessa*, and the flounder, *Platichthys flesus*, in the West Baltic and South Baltic.

Between 1902 and 1920, female plaice in the southern Baltic matured at 3 yr at a length of 16 cm (Molander 1955). The situation was still much the same in 1922 although a slight increase in length at maturity may have occurred from 16 to 16.8 cm (Molander 1955). By 1955, however, female plaice were over 30 cm in length at maturity although their age at maturity was still 3 yr (Molander 1955).

A slightly different situation occurred in the plaice stock of the West Baltic. Between 1904 and 1910, females of this stock matured at 3 yr at a length of 22 cm (Johansen 1929). By 1927−28 the length at first maturity had risen to 28 cm (Johansen 1929) and by 1946 it reached 32 cm (Kandler and Thurow 1959). Most significantly, although most fish matured at 3 yr a few 2-yr-olds were mature. However, by 1956−57, 33% of all 2-yr-olds were mature. The growth rate had not changed during the period 1946−56 and the decrease in the length at maturity was solely due to the decrease in the age at maturity. As predicted, the initial change in reproductive parameters was an increase in the length at maturity. Continued selection eventually produced significant

TABLE 4. Reproductive parameters of some species of flatfish.

Species	Egg diam, mm	Fecundity a	Constants B	Maturity L_1	Range L_2	References[c]
Hippoglossoides platessoides Nfld.	3.00	0.32	3.17	36	70	1, 2
H. platessoides Scotland[a]	2.01	0.93	2.97	15	30	3, 4, 5
Glyptocephalus cynoglussus Nfld.	1.16	−0.36	3.41	39	65	3, 6, 7
G. cynoglussus Scotland	1.16	0	3.48	20	44	3, 5, 8
Limanda limanda	0.93	0.86	3.45	12	30	3, 9
Platichthys flesus	0.97	−0.46	4.18	25	34	3, 10
Limanda ferruginea New England	0.4	−0.006	3.86	28	54	6, 11
Hippoglossus stenolepis	3.27	−1.64	3.56	80	200	12, 13
Microstomus kitt	1.29	1.10	2.70	24	44	3, 14
Scophthalmus maximus[b]	1.06	1.31	3.00	36	70	3, 15
Pleuronectes platessa	1.92	0	3.27	28	48	3, 5, 16
Pseudopleuronectes americanus	0.81	−0.45	4.06	22	44	17, 18
Microstomus pacificus	2.33	−1.24	3.73	42	64	19, 20
Reinhardtius hippoglossoides	4.00	−4.20	4.66	70	105	21, 22

[a]Several years given, one representative year chosen.
[b]Derived from data in paper, published equation in error.
[c]References 1, Wells 1965; 2, Pitt 1964; 3, Russel 1976; 4, Bagenal 1957; 5, Bagenal 1973; 6, Bigelow and Schroeder 1963; 7, Bowering 1978; 8, Bagenal 1963; 9, Bohl 1957; 10, Hoffman 1971; 11, Howell and Kesler 1977; 12, Novikov 1964; 13, Schmitt and Skud 1978; 14, Newton and Armstrong 1974; 15, Jones 1974; 16, Bagenal 1963b; 17, Klein-MacPhee 1978; 18, Kennedy and Steele 1971; 19, Hagerman 1952; 20, Harry 1959; 21, Smidt 1969; 22, Lear 1970.

numbers of fish whose oocyte maturation time was reduced and there was a decrease in the age at maturity, with a concomitant decrease in the length at maturity, a decrease, however, due entirely to the shift in the age at maturity and not a shift in growth rate.

Another species found in the Baltic, and heavily fished during the first half of this century, is the flounder. As with the plaice of the southern Baltic a significant increase in the growth rate occurred during this period. By 1932 flounders on the Oder Bank area of the Baltic became reproductively mature 1 yr earlier than in 1925, 'the majority of the females at the end of the fourth year, for a great part of the males already at the end of the third year' (Kändler 1932). Molander (1955) reporting on the southern Baltic stock, which includes the Oder Bank area, presents a somewhat different situation, which, because of its importance, I shall report verbatim: 'During the years preceding the introduction of up-to-date fishing spawning males were observed measuring 13−15 cm in length. At the end of the twenties and beginning of the thirties I found spawning females 22 cm in length. There were no flounders under three years of age that were ready to spawn and mature females 22−23 cm in length were obtained. However, both males and females are mostly larger at the first spawning. *Thus the great alteration in the growth of the flounder has not involved any corresponding change in the age at which the flounder spawns*' (Molander's italics). Two points should be noted; firstly the period referred to by Molander is somewhat later than that described by Kändler and secondly Molander considers the minimum age at first reproduction while Kändler refers to the age at which the majority of a particular sex are mature. Taking these facts into account and the growth data for the period 1925−32 and qualitative observations for the earlier part of this century given by Blegvad (1932), I propose that the sequence of events has been as follows. For some reason, be it fishing, climatic changes, or a combination of these or other factors, the rate of growth of flounders increased in the present century. This increase in growth rate meant that fish were reaching sizes at which they were formally sexually mature at an earlier age. Previously most females matured at 5 yr of age with some maturing at 3 yr. With the increased growth rate most fish were able to mature one year earlier but genetic/physiological constraints prevented the minimum age at maturity from declining further. Eventually most fish were running up against this physiological barrier and hence the size at maturity increased during the period described by Molander. That this barrier is not an ultimate one, at least for males, is suggested by the occurrence of sexually mature 2-yr-old flounder in Loch Craiglin in Scotland (Gross 1949), and the West Baltic (Kändler and Thurow 1959).

On the Question of Allocation of Energy to Reproduction

The common factor in the analyses so far is the trade-off between reproduction, growth, and survival. In this section I consider how flatfish that are mature allocate their reserves to reproduction.

It is a common observation in fish, and in poikilotherms in general, that fecundity increases as a power function of body length, the exponent generally being about 3 (Bagenal 1966, 1978; Wootton 1979). The reasons why are not readily apparent but the approximately cubic relationship suggests that body volume may be an important factor. A simple hypothesis is that the space within a fish that can be allocated to eggs is limited. A refinement of this hypothesis is that it is not the space that can be allocated to eggs that is limiting but the size of the organs in which the energy is stored, to be converted into gonadal tissue during winter. Indeed it is reasonable to suppose that storage tissues such as the liver maintain an

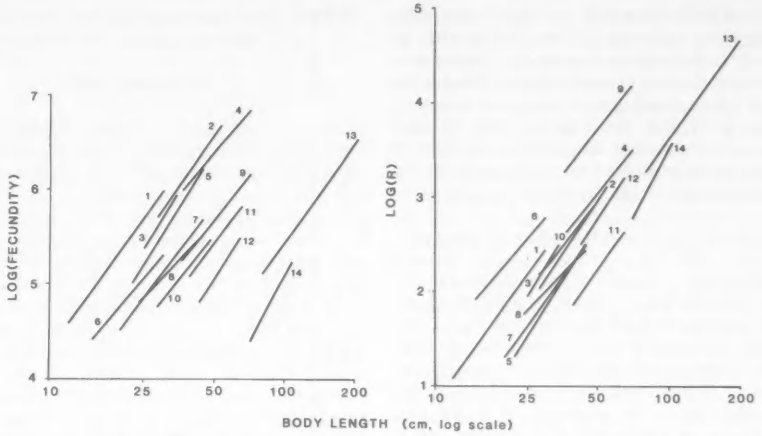

FIG. 2. (*Left*) Fecundity as a function of length in various species of flatfish. (*Right*) Reproductive material as a function of length in various species of flatfish.

1. *Limanda limanda*
2. *Limanda ferruginea*
3. *Platichthys flesus*
4. *Scophthalmus maximus*
5. *Pseudopleuronectes americanus*
6. *Hippoglossoides platessoides* (Scotland)
7. *Glyptocephalus cynoglossus* (Nfld.)
8. *Microstomus kitt*
9. *Hippoglossoides platessoides* (Nfld.)
10. *Pleuronectes platessa*
11. *Glyptocephalus cynoglossus* (Scotland)
12. *Microstomus pacificus*
13. *Hippoglossus stenolepis*
14. *Reinhardtius hippoglossoides*

allometric relationship with the overall body dimensions. An underlying assumption of the above hypothesis is that either fish can obtain all the energy that they require or that energy acquisition is size dependent.

Given an excess amount of food, one would expect a fish to store up as much energy as possible in the liver and other storage tissues. In the hypothesis previously advanced, the amount that can be stored by some populations of flatfish is size dependent to the extent that below a certain size a female cannot produce eggs without incurring a risk of mortality that will be strongly selected against. With the caveat outlined in the previous section, maturity is more likely to be related to size than age, this size being selected through the trade-off between reproduction, growth, and mortality. Selection will optimize the allocation at a particular size within these two constraints. This optimization is a relatively long-term phenomenon operating over many generations. In the short term a fish must allocate energy to gonad production in response to present conditions. Changes in predator abundance are unlikely to play a significant role in this 'fine tuning' of allocation pattern; of greatest significance will be the amount of energy that can be stored and the amount required for maintenance during the winter months. There are two extremes in the pattern of allocation. Firstly, a fish may maintain a constant body weight over time and adjust egg or sperm production accordingly. Secondly, a fish may produce a constant number of eggs and sacrifice body tissues to meet the requirements of production. Within these two limits a fish might, for example, sacrifice egg production at a rate that increases with decreasing body condition. Several studies have shown that starvation reduces fecundity (Scott 1962; Hester 1964; Bagenal 1969; De Vlaming 1971; Tyler and Dunn 1976; Wootton and Evans 1976; Wootton 1977). There are no species reported which adopt either extreme pattern, but the threespine stickleback *Gasterosteus aculeatus* represents the type that sacrifices body condition to maintain egg production (Wootton and Evans 1976; Wootton 1977), whereas the winter flounder is closer to the other extreme, sacrificing egg production and maintaining body weight (Tyler and Dunn 1976). The considerable jellying of the flesh in American plaice off Newfoundland indicates that they are sacrificing considerable somatic tissue to produce eggs. Furthermore, both the growth rate and the increase in the percentage of jellied plaice with length varies between areas (Pitt 1967; Templeman and Andrews 1956), but the fecundity/length function does not (Pitt 1964). This suggests that body condition is sacrificed to maintain egg production. A jellied condition of the flesh has not been reported in the much-studied plaice *Pleuronectes platessa* although it undergoes a winter fast (Dawes 1930, 1931; Wimpenny 1953; Lande 1973). This suggests that body condition in this species is maintained at the expense of egg production. Such a strategy would explain the unusually long reproductive life span of this species (26 yr, Roff 1981).

There are insufficient data to be able to arrange a significant number of the flatfish species (or indeed any group) along the allocation spectrum described above. However, data on the fecundity/length function between different species can shed light upon the degree of variation in allocation to reproductive material. If there is a strong allometric constraint on fecundity, we may expect to find the fecundity/length functions to be very similar. Similarity might, however, also arise because

of similarities in natural histories: this latter hypothesis can be rejected by using species with very different life histories. In assessing reproductive expenditure we must take into consideration differences in egg size between species. Data on the fecundity/length functions and egg size for a wide variety of flatfish are given in Table 4. In all but one case, *Pseudopleuronectes americanus*, the eggs are pelagic. A measure of the amount of material committed to reproduction can be obtained by multiplying the fecundity by the egg volume,

$$(1)\quad \text{Log } R = \left(\log \pi \frac{D^3}{8}\right) + a + \text{B log } L = A + B \log \text{L}$$

where R is the reproductive material, D is the diameter of the egg, L the length of the fish, and a and B the constants of the fecundity/length function. As can be seen from Fig. 2, the correction for egg volume considerably reduces the variation between species. There is a highly significant negative correlation between the two parameters 'A' and 'B' ($r = 0.92$, $n = 14$, $P < 0.001$, Fig. 3). The parameters 'A' and 'B' are of considerable biological significance. If B is greater than 3 the allocation to reproduction relative to body weight increases with weight; if B is less than 3 the allocation to reproduction decreases relatively with size. Thus, for example, Greenland halibut (*Reinhardtius hippoglossoides*) (14 in Fig. 3) appear to increase their commitment to reproduction at a very much higher rate than *Microstomus kitt* (8 in Fig. 3). However, the proportionality constant A in these two species is different, being small in the former species and large in the latter. Before seeking biological explanations for these differences, it is necessary to examine the statistical dependence between A and B. This can be done using covariance analysis. Unfortunately the raw data on all species are not available: I therefore compared two species at either extreme, the flounder and American plaice (see Fig. 3). There are several years of data for American plaice and I chose 1958 as a representative year (data from Bagenal 1965). The analysis of covariance indicates that there is no significant difference in slope ($F_{1,100} = 2.59$, $P < 0.05$) but a significant difference in the intercept ($F_{1,100} = 174$, $P < 0.001$). Thus, at least part of the correlation between A and B is ascribable to a statistical dependence of the two parameters. However, there also appears to be a biological component: Bowering (1980) reports a significant difference in B between Greenland halibut taken in Southern Labrador and southeastern Gulf of St. Lawrence. Significantly the values of A and B for the Southern Labrador stock are quite different from those obtained from Lear (1970) (Fig. 3): unfortunately Bowering did not statistically compare the two sets of data and it is not therefore possible to say whether these differences are significant either statistically or biologically. Although there may be biologically meaningful differences in B, the differences are not as great as the variation in A (Fig. 2) and it is possible to rank the species based on fecundity or reproductive volume (R). There is a nonsignificant correlation between these rankings (Spearmans rank correlation, $r_s = 0.525$, $t = 2.138$, $P > 0.05$, but there is a trend for species to have a similar rank on both measures (for a significant correlation we require $t = 2.18$). The low correlation between the rankings clearly indicates that fecundity per se is not a reliable indicator of reproductive effort.

In American plaice from Scottish waters the fecundity/length relationship varies between years (Bagenal 1957, 1965). Covariance analysis shows that this variation is due to changes in the intercept 'a' ($F_{8,317} = 2.34$, $P < 0.05$) and not the slope 'B' ($F_{7,317} = 1.80$, $P > 0.5$). Thus, the relative allocation to reproduction is the same for fish of different sizes, but the actual amount allocated may vary. Although the year-to-year variation is statistically significant, its contribution to the explained variance is very slight (an increase from 75.1 to 76.3%) and this variation is of little consequence relative to the length component (which by itself accounts for 75.1%). Biologically it may be that in this stock slight adjustments are made each year according to environmental conditions or it may result from variations in the time of sampling.

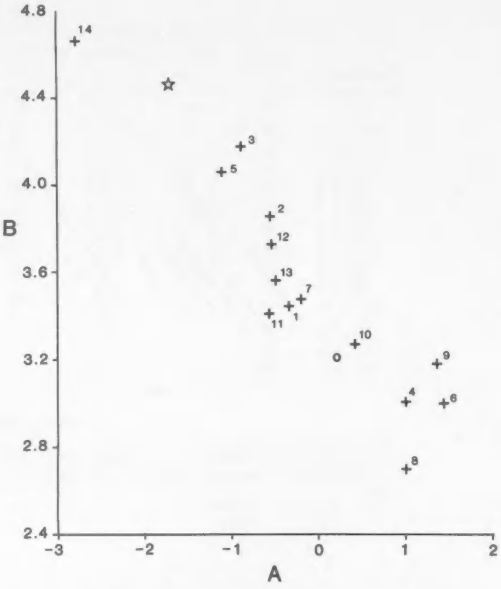

FIG. 3. Plot of the two parameters 'A' and 'B' from the function Log (R) = A + B Log (length). (○), estimates of Bowering (1980) for Southern Labrador; (☆), Bowering's estimate for Gulf of St. Lawrence stock.

What factors might cause the pattern of allocation to reproduction to vary? The most obvious factor is variability in the food supply. An increase in food increases the amount of energy available for egg production. How this is reflected in the parameters of the fecundity function will depend upon whether fish of all sizes can take advantage of this increase. Suppose, for example, there was an increase only in large-sized prey: only those fish large enough to take these items will show an increased fecundity. This differential effect will appear as an increase in the slope (B) of the fecundity function. If fish of all sizes can take advantage of the increased food supply, the slope will remain constant but the intercept (a) will increase. The same argument applies also between species. Variation in A and B may reflect variation in the food supply potentially available to a species. An analysis of the

ecological and/or physiological factors determining these parameter values would be of considerable value.

Discussion

The onset of maturity may depend on size or age. Age appears to be most important in species that mature early. This dependence is predictable given the relationship between the rate of increase r and generation length. It is predicted that environmental changes will, initially at least, affect the size at maturity in early maturing species and the age of maturity in late maturing species. Changes in the reproductive parameters of the North Sea sole, Baltic plaice, and Baltic flounder support the hypothesis of a genetic/physiological barrier in early maturing species, in all cases the size at maturity changing. American plaice off Newfoundland demonstrate that substantial shifts in the age, but not size, of maturity can occur in a late maturing species. However, the aforementioned species are extreme cases, maturing either very early (2−3 yr) or very late (12−14 yr). There are many species midway in this range and it is to be expected that there will be species in which maturity depends on both age and size. In this regard changes in the maturity parameters of the yellowfin sole *L. aspera* of the west Kamchatka shelf reported by Tikhonov (1978) are particularly interesting. In samples collected between 1961 and 1963, the mean age at maturity was 8.5 yr with a range from 5 to 13 yr while the average length at maturity was 29.9 cm with a range from 20 to 35 cm. By the period 1967−69, the mean age at maturity had dropped to 7.2 yr. Although the minimum age at maturity was still 5 yr, the percentage mature at this age had risen from ~2 to 15%. The average length at maturity had declined to 27.2 cm and the minimum length to about 17 cm. The large range in the age of maturity indicates that the population can respond to changes favoring a reduction in the age at maturity. Thus, if, as might be hypothesized, selection favored a reduced size at maturity between 1961 and 1969, there already existed in the population individuals that could respond to this pressure by maturing at an earlier age and hence smaller size. The failure of individuals less than 5 yr old to mature is not surprising given the initially low percentage of individuals capable of maturing at this age. Tikhonov (1978) also reports an increase in fecundity during this time period but because both the intercept (a) and the slope (B) of the fecundity function change and no statistical comparison is made, this conclusion requires verification.

Unfortunately there is no direct evidence of the causes of the observed changes in reproductive parameters. De Veen (1970, 1976) suggests that trawling has made food more abundant, and this is the probable cause of changes in the life history of the North Sea sole. Trawls, particularly the tickler chains of the beam trawls, may cause considerable damage to the sea bed and invertebrates (ICES 1971, 1972, 1973). The effect of the trawl is to drag invertebrates out of the mud, in many cases causing considerable damage to them (Bridger 1970; De Groot and Apeldoorn 1971) thereby making them easy prey. Underwater observations suggest that dab congregate in the path of the trawl actively searching for food (Margetts and Bridger 1971), a phenomenon reminiscent of gulls following a ploughman. De Veen (1976) reports unpublished observations of Bannister and Lee of 'the occurrence of the gonads of Echinocardium in the stomach contents of the dab in the North Sea, an unlikely occurrence if the Echinocardium had not already been damaged by a gear.' In the North Sea fishery, dutch beam trawlers were first introduced in 1962 and the number of hours fished by this gear rapidly increased from 97 000 h in 1962 to a maximum of 1 015 000 h in 1967, thereafter fluctuating at about 800 000 h.

The changes in the reproductive parameters of the North Sea sole were first observed in 1963 and hence there is circumstantial evidence that the increase in fishing may be responsible. Kändler (1932) and Molander (1938) attributed the changes observed in the flounder of the southern Baltic to a decrease in density resulting from heavy fishing. However, Belgvad (1932) demonstrated that temperature may significantly affect growth rate in flounders and it is possible that environmental changes other than fishing may be responsible for the increased growth rate of the flounder. The changes in the maturity schedule of the Newfoundland American plaice are also coincident with increased fishing pressure (Pitt 1975). In all cases the changes in the onset of maturity have been those that might be expected to occur when conditions experienced by individuals improved; for example, if food became relatively more abundant as a result of decreased density or increased temperatures.

The relationship between fecundity and size may favor further growth in female fish after the onset of maturity, but such an argument can hardly apply to male fish. But male fish also continue to grow after maturity although their rate of growth is generally less than that of the immature females. The conventional wisdom seems to be that the reduction in growth rate of the males is due to the allocation of resources to the testes. However, the amount allocated to the gonads is rather small and seems hardly sufficient to have a great impact on growth. Why should a male fish grow after maturity? In some species of fish, males may actively compete for females and size may be of advantage is these contests. Data on the reproductive behavior of flatfish is scarce but I can find none that suggests any fighting for females. An increase in size may reduce the probability of being preyed upon and thus might be a factor favoring continued growth in males. However, growth requires energy and this means foraging for food. Increased foraging probably increases the risks of predation and therefore there will be a trade-off between the amount of time spent foraging to grow bigger and avoid predation by size versus the avoidance of predation by remaining inactive. I suggest that having reached the size at which they can successfully reproduce, the growth of male flatfish declines in part as a response to the divergence of energy into reproduction, and, in part, as a response to decreased foraging activity.

Acknowledgments

I am most grateful to Drs D. J. Fairbairn, G. Lilly, W. Leggett, and M. Lechowicz for their careful and critical reading of earlier drafts of this paper. Dr Al Tyler very kindly supplied me with unpublished data. I am thankful to Dr T. Pitt and Mr R. Bowering for helpful discussions on the life histories of the Newfoundland American plaice and witch.

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Characteristics of Sediment and Organic Carbon Export from Pristine Boreal Forest Watersheds[1]

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NAIMAN, R. J. 1982. Characteristics of sediment and organic carbon export from pristine boreal forest watersheds. Can. J. Fish. Aquat. Sci. 39: 1699–1718.

Estimates of the amount of material moving annually from terrestrial ecosystems to the ocean are largely based on an incomplete understanding of events occurring throughout the hydrologic year, and only a vague comprehension of in-stream processes controlling that export. Discharge, suspended sediment, particulate organic matter (POM; >0.5 μm), dissolved organic carbon (DOC; <0.5 μm diameter), and the percentage of organic matter were measured from 1979 to 1981 in five pristine Quebec streams: First Choice Creek (1st order; watershed area: 0.25 km^2), Beaver Creek (2nd order; 1.83 km^2), Muskrat River (5th order; 204 km^2), Matamek River (6th order; 673 km^2), and the Moisie River (9th order; 19 871 km^2). All streams, with the exception of First Choice Creek, have a strong spring freshet when 43–55% of the annual discharge occurs. By describing sediment and organic carbon export throughout the annual hydrologic cycle, I showed that during the 2-mo spring freshet 71–92% of the annual sediment load is exported but only 59–65% of the annual POM load, and only 47–51% of the annual DOC load. Sediment yield is relatively constant between watersheds (1.5–7.6 $g \cdot m^{-2} \cdot yr^{-1}$), as is POM export (1.0–6.7 g ash-free dry-weight [AFDW]$\cdot m^{-2} \cdot yr^{-1}$); however, export DOC varies from 3.1 g $C \cdot m^{-2} \cdot yr^{-1}$ in First Choice Creek to 48.4 g $C \cdot m^{-2} \cdot yr^{-1}$ in Beaver Creek. There appears to be rapid loading of carbon between 1st- and 2nd-order streams in boreal forests, followed by biological and physical processing as watershed area increases. Thus, for the Moisie River watershed, export of total organic carbon (TOC) is reduced to only 4.7 g $C \cdot m^{-2} \cdot yr^{-1}$. Export of coarse particulate organic matter (>1 mm) is negligible (normally <0.1 $mg \cdot L^{-1}$), as is oxidation of the suspended load (<0.5% $\cdot d^{-1}$). Effects of summer storms, natural diel variations, and depth of sample from the water column are shown to have a minimal influence on concentrations. Rating curves ($kg \cdot d^{-1}$ vs. discharge) are developed to estimate the annual yield of sediment, POM, and DOC, and to evaluate long-term variations. From the results I suggest that in-stream processing and retention devices exert considerable control over the quantity and nature of suspended organic material. Physical processes such as the discharge regime and stream power are relatively less important in determining organic concentrations, but more important in determining sediment concentrations.

Key words: seston, carbon, sediment, boreal forest, watershed, river, stream, export

NAIMAN, R. J. 1982. Characteristics of sediment and organic carbon export from pristine boreal forest watersheds. Can. J. Fish. Aquat. Sci. 39: 1699–1718.

Les estimations de la quantité de matériel transféré annuellement d'écosystèmes terrestres à l'océan reposent en grande partie sur une compréhension incomplète des événements de toute l'année hydrologique et seulement une vague compréhension des processus à l'intérieur des cours d'eau qui contrôlent ce transfert. Le débit, le sédiment en suspension, la matière organique particulaire (POM; >0,5 μm), le carbone organique dissous (DOC; <0,5 μm de diamètre) et le pourcentage de matière organique ont été mesurés entre 1979 et 1981 dans cinq cours d'eau promitifs du Québec : ruisseau First Choice (1er ordre; superficie du bassin hydrographique : 0,25 km^2), ruisseau Castor (2^{e} ordre; 1,83 km^2), rivière aux Rats Musqués (5^{e} ordre; 204 km^2), rivière Matamek (6^{e} ordre; 673 km^2), et rivière Moisie (9^{e} ordre; 19 871 km^2). Tous ces cours d'eau, sauf le ruisseau First Choice, ont de fortes crues printannières, alors que 43 à 55 % du débit annuel se produit. En décrivant l'exportation du sédiment et du carbone organique du cycle hydrologique complet, je démontre que, pendant les crues printannières de 2 mois, 71 à 92 % de la charge de sédiment annuelle est exportée, mais seulement 59–65 % de la charge de POM annuelle et 47–51 % de celle de DOC. Le

[1]Contribution No. 5154 from the Woods Hole Oceanographic Institution and No. 62 from the Institution's Matamek Research Station.

Printed in Canada (J6917)
Imprimé au Canada (J6917)

rendement en sédiment est relativement uniforme d'un bassin hydrographique à l'autre (1,5−7,6 $g \cdot m^{-2} \cdot an^{-1}$), ainsi que l'exportation de POM (1,0−6,7 g de poids sec sans les centres $[AFDW] \cdot m^{-2} \cdot an^{-1}$); cependant, l'exportation de DOC varie de 3,1 g $C \cdot m^{-2} \cdot an^{-1}$ dans le ruisseau First Choice à 48,4 g $C \cdot m^{-2} \cdot an^{-1}$ dans le ruisseau Castor. Il semble y avoir, dans les forêts boréales, un chargement rapide de carbone entre les cours d'eau de 1er et 2^{e} ordre, suivi d'une transformation biologique et physique à mesure qu'augmente la superficie du bassin hydrographique. C'est ainsi que, pour le bassin de la rivière Moisie, l'exportation du carbone organique total (TOC) est réduite à seulement 4,7 g $C \cdot m^{-2} \cdot an^{-1}$). L'exportation de matière organique particulaire grossière (>1 mm) est négligeable (normalement <0,1 $mg \cdot L^{-1}$), tout comme l'oxydation de la charge en suspension (<0,5 % $\cdot d^{-1}$). Nous démontrons que les orages d'été, les variations nycthémérales naturelles et la profondeur à laquelle l'échantillon est prélevé dans la colonne d'eau ont très peu d'influence sur les concentrations. Nous traçons des courbes d'évaluation ($kg \cdot d^{-1}$ vs débit) dans le but d'estimer le rendement annuel en sédiment, POM et DOC, et aussi les variations à long terme. Les résultats obtenus me font croire que la transformation au sein des cours d'eau et les dispositifs de rétention exercent un contrôle considérable sur la quantité et la nature de la matière organique en suspension. Les processus physiques tels que le débit et la puissance du cours d'eau sont relativement moins importants dans la détermination des concentrations organiques, mais plus importants dans celle des concentrations de sédiment.

Received April 27, 1982
Accepted September 7, 1982

Reçu le 27 avril 1982
Accepté le 7 septembre 1982

THE downstream movement of material to the ocean shapes both the geological form of channels as well as the character of biological communities through the amount and nature of materials being carried, and the seasonal timing. In recent years seston has received considerable study, especially relating to estimates of organic and nutrient budgets, but few studies have addressed mechanisms controlling seston export and no comprehensive studies have been conducted on pristine watersheds.

From previous investigations it is known that relatively undisturbed watersheds are highly retentive of organic matter; most organic inputs are released downstream only after being processed to small particles (Sedell et al. 1978; Naiman and Sedell 1979a). Discharge and retention devices interact to influence concentrations carried downstream (Dunne and Leopold 1978; Bilby 1981; Bilby and Likens 1979, 1980), and published data often show only weak correlations between stream flow and organic carbon concentrations (Fisher and Likens 1973; Fisher 1977). Nevertheless, the annual release of materials downstream can be reasonably predicted as a function of watershed area for various biome types (Schlesinger and Melack 1981; Mulholland and Watts 1982) because most of material export occurs during intense storm events or during spring freshet (Bormann et al. 1974).

The effect of discharge on particulate and dissolved materials has been adequately demonstrated, although complete dependence of single variable on hydrology has seldom been documented (Bormann et al. 1974; Hobbie and Likens 1973; Sedell et al. 1978; Naiman and Sedell 1979a; Bilby and Likens 1980). Once discharge decreases below a threshold value it is thought materials accrue within the system (i.e. export < import); however, when discharge exceeds some threshold, materials are entrained and transported downstream. As Naiman and Sedell (1979b) discussed, the interaction between hydraulic power and in-stream retention devices influences the amount of material transported relative to that available for transport, and the relative proportions of size-classes moved. It is assumed that each retention device has some threshold which can be overcome by hydraulic power, rendering it ineffective for holding material within the system. Therefore, knowledge of the long-term and short-term hydrologic regime, and the nature of precipitation, soils, and retention devices is essential for understanding export patterns. Unfortunately, the relative roles of the numerous retention devices in this process cannot yet be quantified.

Most concepts of stream sediment and organic carbon transport have been developed in temperate regions, and usually on small watersheds with a history of disturbance. This study examines transport parameters on five streams ranging in size from 1st to 9th order in the boreal forest of eastern Canada. Sediment (>0.5 μm), particulate organic matter (POM >0.5 μm), dissolved organic carbon (DOC <0.5 μm), and the percentage of organic matter (% OM) in the seston were measured over a 3-yr period starting each year before ice breakup and continuing to ice formation.

This study has important theoretical and applied implications. For the first time concepts concerning the export pattern of materials are tested on large rivers throughout the annual hydrologic cycle, and the effect of the spring flood on export characteristics is examined in detail. Concentrations of suspended materials and discharge are used to examine processes controlling export and to estimate mass movement of materials. Reliable estimates of annual export are now available from the boreal forest region, and export characteristics from pristine watersheds can be compared to studies conducted on previously perturbed systems.

Study Areas

The study sites are located about 25 km east of Sept Iles, Quebec, on the North Shore of the Gulf of St. Lawrence. This region is Precambrian Shield characterized by low gradient streams (<2% slope) and waters colored by humic acids (DOC; 5−15 mg $C \cdot L^{-1}$). All streams experience a strong

TABLE 1. Physical and chemical characterization of the Quebec study sites [modified from Naiman (1983)].

Parameter	First Choice Creek	Beaver Creek	Muskrat River	Matamek River	Moisie River
Stream order	1	2	5	6	9
Watershed area (km^2)	0.25	1.83	207	673	19,871
Mean width (m)	0.3	2.0	21.9	51.7	208.7
Mean depth (m)	0.05	0.3	2.0	1.8	2.5
Mean annual discharge ($m^3 \cdot s^{-1}$)	0.013	0.033	8.4	13.7	466.1
Gradient (%; lower reaches)	1.5	1.0	0.14	1.33	0.16
Substrate	Detritus	Sediment/cobble	Sediment/cobble	Gravel/cobble	Sand/cobble
Temperature range (°C)	0.4–11.0	0.0–19.0	0.0–19.5	0.1–19.8	0.1–21.8
Annual degree days ($°C \cdot yr^{-1}$)	1702	2069	2157	2225	2219
Nutrient range ($\mu g \cdot L^{-1}$)					
NO_3-N	17–199	5–135	3–91	1–76	25–87
Total N	49–234	211–623	170–382	148–341	134–392
PO_4-P	2–8	1–7	1–5	1–17	1–11
Total P	7–29	6–26	6–22	5–16	4–22
pH range	6.2–7.2	6.0–7.0	4.9–6.1	4.8–6.0	6.3–7.1
Mean dissolved organic carbon ($mg\ C \cdot L^{-1}$)	4.1	12.9	12.3	11.3	6.8
Forest canopy development	Closed	Mostly closed	Mostly open	Open	Open
Benthic organic matter ($g\ AFDW \cdot m^{-2}$)	712	526	516	252	481

spring freshet in April–May. Extensive snow and ice pack are common from November to late April; flows in January–February are only 3–5% of the annual maximum. Dominant forest vegetation is black and white spruce (*Picea mariana* and *P. glauca*; 47% of the area) and balsam fir (*Abies balsamea*; 44%). Paper birch (*Betula paprifera*), speckled alder (*Alnus rugosa*), and trembling aspen (*Populus tremuloides*) form the dominant stream-side vegetation. Four of the five sites (First Choice Creek, Beaver Creek, Muskrat River, Matamek River) are within the Matamek River Watershed, protected by the Province of Quebec for scientific investigations and controlled fur trapping only. The fifth site (Moisie River) is adjacent to the Matamek River Watershed (Table 1, Fig. 1). Various aspects of these ecosystems have been previously described (e.g. Power et al. 1973; Naiman and Sedell 1981; Naiman 1980, 1981a, 1982, 1983).

First Choice Creek begins as a series of seeps (Table 1, Fig. 1). Most organic inputs originate from the closely associated forest as leaves, needles, and wood falling directly into the stream, and occasionally from lateral inputs originating from herbaceous plants such as ferns (*Dennstaedtia punctilobula*).

Beaver Creek is essentially a series of abandoned beaver dams and ponds interspersed between long riffles (Table 1, Fig. 1). Major organic inputs are wood from previous beaver activity, leaves, some periphyton production, and organic material transported from upland tributaries.

The Muskrat River originates in an upland area but also receives the discharge of a large muskeg with highly colored, slowly flowing water (Table 1, Fig. 1). Typical river morphology is that of a deep and narrow channel meandering between widely spaced waterfalls. Primary production by diatoms, filamentous algae, and mosses, as well as organic matter transported from upstream areas are the major organic inputs. In summer and autumn black spruce, balsam fir, paper birch, and speckled alder provide some allochthonous material. The Muskrat River is a major tributary of the Matamek River.

The Matamek River, unlike the other sites, is physically heterogeneous (Table 1, Fig. 1). A large lake (16.2 ha) in its lower reaches ameliorates the discharge pattern, modifying some ecological characteristics. Morphologically, it is a stair-step river from the lake to the Gulf of St. Lawrence (~7 km) with five waterfalls separating long sections of turbulent rapids and moderately swift water. Alder, birch, and conifers contribute some material, but most organic matter is synthesized by periphyton, mosses, extensive beds of macrophytes, and material transported from upstream areas.

The Moisie River is a broad river draining into the Gulf of St. Lawrence (Table 1, Fig. 1). Despite the large size of the watershed there has been a minimal amount of human disturbance. There is a single iron mine in the headwaters of one tributary (Nipissis River) and a railroad transversing the region. Woody debris and fine detritus are relatively scarce in this river. Ice scour during powerful spring freshets move even the largest materials downstream or deposit them high up on the bank. The water elevation during each year varies ~10 m from spring freshet to mean low water. Most in situ organic matter is produced by periphyton, moss, and macrophytes, or originates from material being carried downstream. Allochthonous inputs from alder and other terrestrial species are minor.

Autochthonous and allochthonous inputs, the immediate sources of drifting organic matter, vary widely in amount and composition for each study site. Once inputs reach the stream they have the potential to drift immediately or be retained and processed in place. Each biological input shows a different propensity for drifting and abiotic–biotic processing depending upon biochemical composition, temperature, water chemistry, flow regime, and stream morphology. In general,

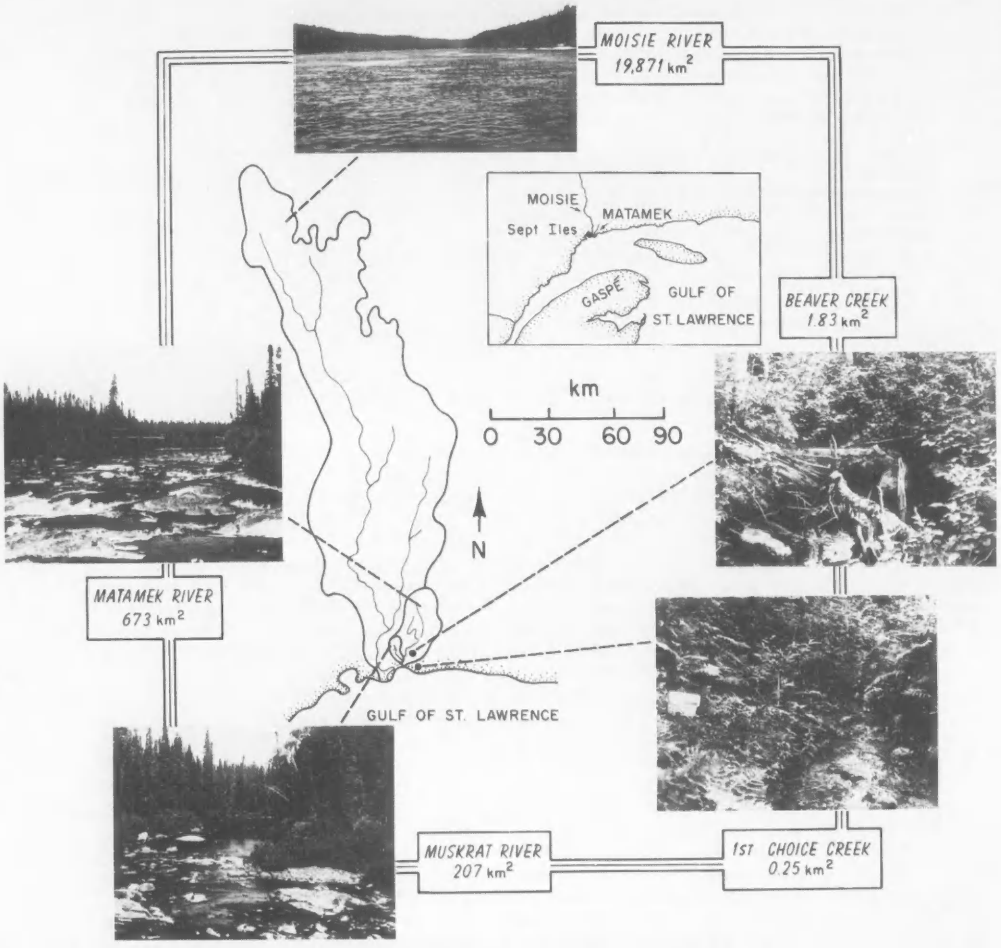

FIG. 1. Location of the five studied watersheds in eastern Quebec, Canada, with photographs of the sites.

all sites have similar temperature regimes with long periods in winter (6 mo) of subfreezing temperatures and short periods (3–4 wk) in summer when maxima of 18–22°C are realized. All sites are quite different in stream flow, channel geometry, and basin morphometry, but as a whole they are a typical continuum as streams gradually coalesce to form rivers.

PRECIPITATION AND SOILS

Precipitation and air temperature strongly influence the hydraulic regime, especially in this region where the mean annual temperature is 1°C and mean monthly minimum temperatures are >0°C only from May to October. Precipitation is evenly distributed throughout the year, normally averaging 6–10 cm per month (Naiman 1980). Most precipitation between mid-September and May occurs as snow with a maximum of ~100 cm deposited in January. There is little melting until late April when, with rapidly increasing air temperature, the snow disappears over a 4- to 6-wk period. Rain dominates throughout the summer, averaging ~10 cm per month.

Long-term cycles in precipitation patterns are well known and significantly influence the storage, movement, and processing of organic matter in watersheds. In this region

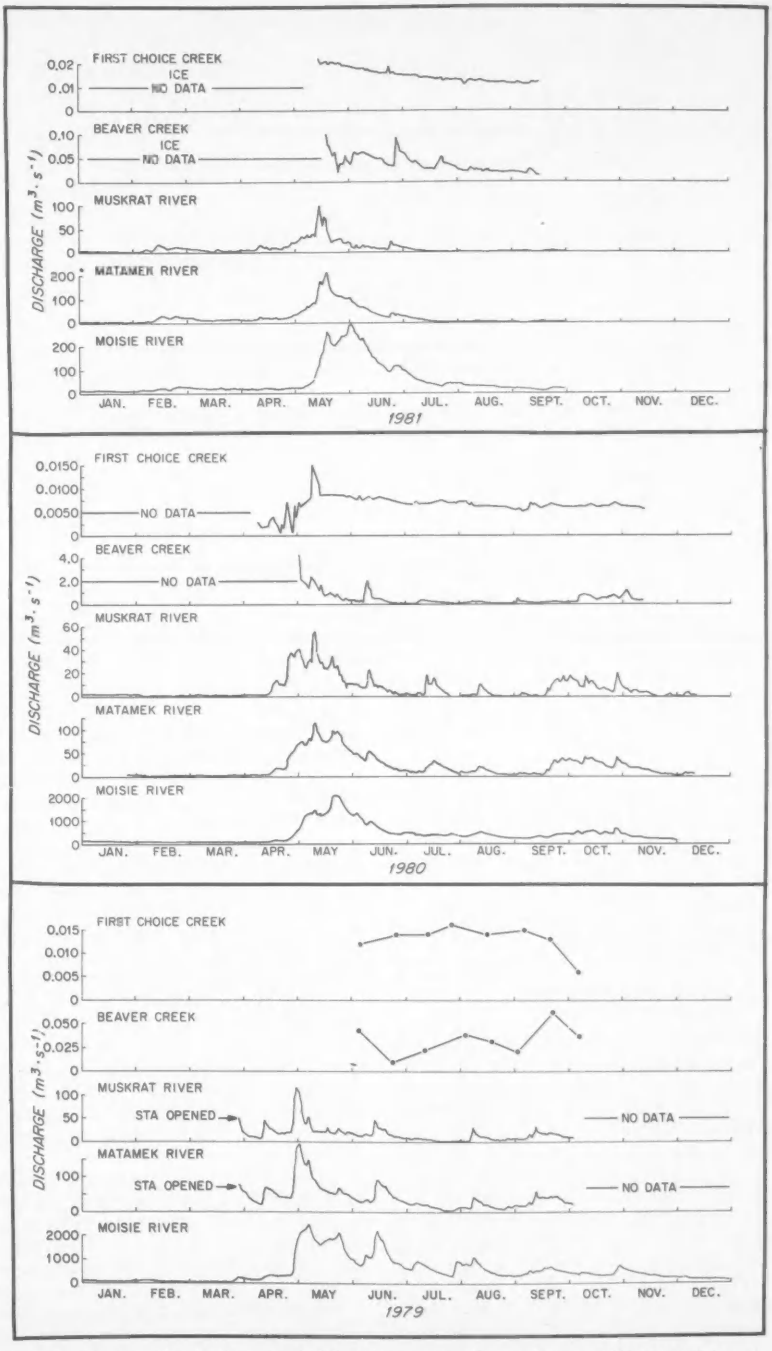

FIG. 2. Discharge from the five watersheds had the same general pattern between 1979 and 1981. In each year there was a strong spring freshet with a secondary freshet in late summer or autumn.

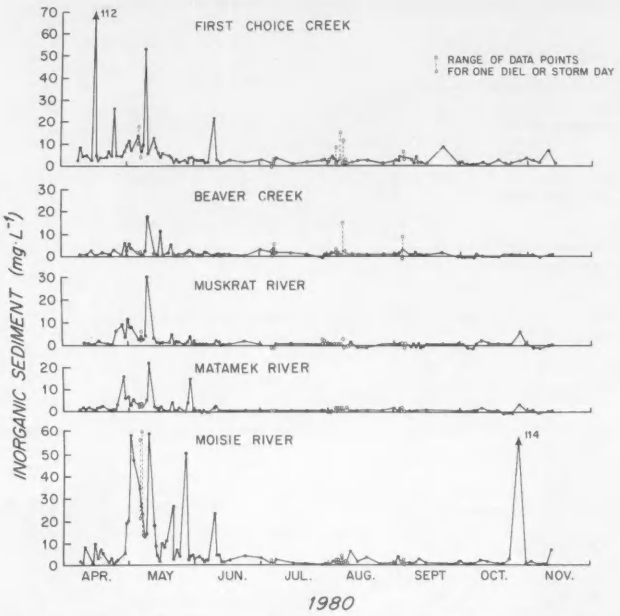

FIG. 3. The annual pattern of the suspended inorganic sediment concentration is shown for each site. Note that highest concentrations are during the spring freshet with considerable day-to-day variability at that time, and that summer and autumn concentrations are negligible.

precipitation is highly variable from month to month, but since 1944 there has been no consistent trend for precipitation to increase or decrease (Naiman 1980).

The interaction between soils and precipitation determines whether runoff erodes the landscape, as when overland runoff occurs, or percolates downward into the groundwater pool but leaving the particulate component intact. Soils are porous podzol with a typical thickness of about 20 cm, overlying the granitic rocks of Precambrian Shield (Frenette and Julien 1981). Most snowmelt and rain water percolates to a hard substrate and eventually seeps into stream channels, rather than entering by erosive overland flow.

Methods and Materials

From April to November between 1979 and 1981, 1-L water samples were collected regularly from the base of waterfalls or well-mixed riffles at all sites, except the Moisie River. In that case water was collected ~0.5 m below the surface in midchannel. Frequency of sampling varied from year to year but was almost daily during freshets and about once every 1−2 wk during periods of relatively uniform discharge. Samples were concentrated onto preashed and preweighed Gelman AE glass fiber filters, and the invertebrates removed. The filter with the sample was then dried at 80°C, weighed, ashed at 525°C for 2−4 h, and weighed again on a Cahn 25 electrobalance. POM concentrations in milligrams ash-free dry-weight per litre (mg AFDW·L^{-1}) were calculated by dividing the AFDW of the sample by the volume of water filtered. AFDW was converted to carbon, assuming organic matter is 50% carbon. Suspended sediment concentrations were calculated by dividing the ash weight of the sample by the volume of water filtered. Invertebrate biomass constituted <1% of the exported carbon, and it is not considered further.

To facilitate comparison with other studies, coarse particulate organic matter (CPOM) is defined as particles >1 mm in diameter; fine particulate organic matter (FPOM) as particles 53 μm−1 mm; very fine particulate organic matter (VPOM) as 0.5−53 μm; and dissolved organic matter (DOM) as <0.5 μm (Boling et al. 1975). On several occasions the relative proportion of particulate size groups was measured by pumping a large volume of water (~10^5 L) through series of sieves with mesh sizes of 1 mm and 53 μm. Water passing through the 53-μm screen was filtered onto a preashed Gelman filter. All samples were then dried and weighed.

Dissolved organic carbon was measured by collecting 50 mL of water passing through the preashed Gelman filter, preserving with 2% phosphoric acid, and analyzing on a Dohrman model 54 carbon analyzer.

Because samples could not be collected from a completely mixed body of water, a cross section of the Moisie River was sampled in a matrix array once a month to examine distribution of suspended sediment and POM concentrations. In a uniformly flowing section, four equidistant stations were established and samples taken at four depths from the surface to ~5 cm from the bottom. The substrate at this site is cemented cobble. Samples were then treated as previously described.

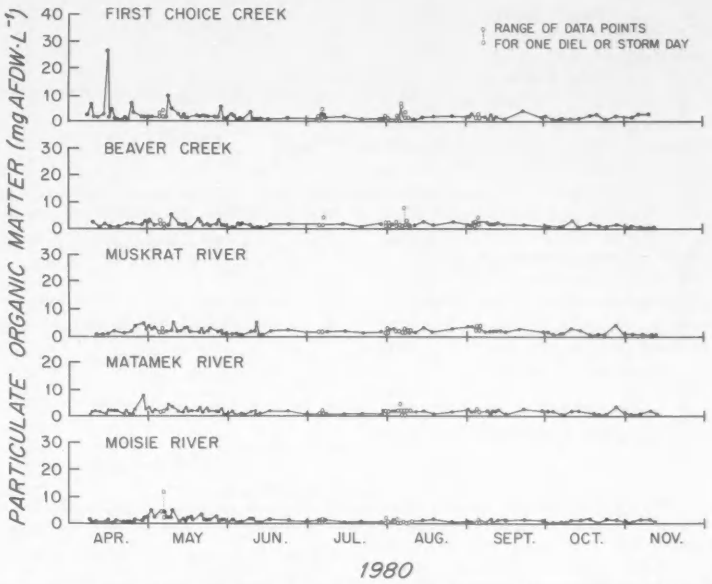

FIG. 4. The annual cycle of suspended particulate organic matter is shown for each site. Note the small increase during the spring freshet; the scale is identical with that in Fig. 3. AFDW is ash-free dry weight.

To examine CPOM export, relative to VPOM, FPOM, and DOC export, stream drift samplers were modified with large nets of 1-mm mesh (mouth opening 30 × 45 cm), and allowed to collect for 24 h during autumn leaf fall. Water flow through the net was measured several times during this period with dyes, and the total volume filtered calculated. In the large rivers the nets were set ~0.3 m below the surface.

Diel variations in the concentration of suspended sediment and carbon were examined simultaneously at each site on four dates. Samples were collected every 3 h over 24 h, and the mean concentration and coefficient of variation calculated.

To determine the variation due to handling or procedure, relative to natural changes in concentration, separate measurements were made on a single water mass. On April 10, 1980, ~40 L of water were collected from the Matamek River, mixed well, and 10 subsamples withdrawn for sediment and POM measurement.

In August 1980, samples were collected every 3 h over 6 d at each site to examine the effects of intense summer storms on export. Sampling started 6 h before the initial rainfall and continued until the hydrographs returned to stable levels.

Discharge in First Choice Creek was recorded daily with a calibrated Parshall flume. In Beaver Creek the flume was improperly installed and destroyed by a flood ~10 d later. For the remainder of the study, discharge was estimated by injecting fluorescent dye into a narrow section of the creek previously charted for depth, width, and volume at various water stages, and the discharge estimated from the time taken for dye to traverse the area. The Matamek River has recording stations operated by the Quebec Ministry of Natural Resources above and below the confluence of Muskrat River; the difference being the Muskrat discharge. The Moisie River has a recording station within 2 km of the sampling site.

The amount of material transported by streams is partially a function of discharge and stream power. Unit stream power is an indication of the ability of water to overcome retention devices and move particles, and can be estimated from the equation $\omega = \rho Qs/w$ where ω is power per unit width ($kg \cdot m^{-1} \cdot s^{-1}$), Q is discharge ($m^3 \cdot s^{-1}$), s is slope in (%), w is width in metres (m), and ρ is the density of water (Leopold et al. 1964). This is the work rate of flowing water per unit width of stream.

Results and Discussion

HYDROLOGY

The discharge patterns — Winter temperatures freeze streams almost solid for nearly 3 mo; thus, discharge for all sites is minimal between January and March (Fig. 2). With thawing in April, discharge increases from near the annual minimum to the annual maximum over a 5- to 10-d period. The 0.6–1.2 m of ice cover is rapidly broken, and jumbled ice floes scour channels for about a week. The spring freshet continues until late May or mid-June, depending upon the size of the watershed, with peak flooding occurring earlier in small streams. After the spring flood, flows are relatively constant in 1st- to 3rd-order streams, but in the larger rivers, except for brief storms, flows gradually decrease over the summer. By

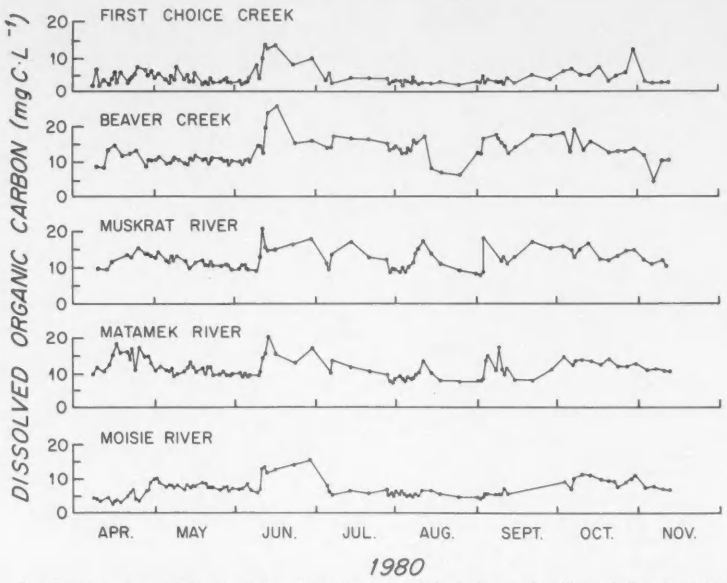

FIG. 5. Concentrations of dissolved organic carbon are relatively low and uniform during the spring freshet but increase during the productive summer and autumn months. Note that all sites show a similar pattern.

autumn, discharge is generally low and constant at all sites. Occasionally there will be secondary freshets in October associated with autumn rains.

During the 1980 spring freshet 43–55% of the annual discharge occurred during a 2-mo period from April 15 to June 14, except in First Choice Creek where only 24% of the annual discharge occurred during the same period. The pattern in rivers is similar for previous years where data are available. For example, since 1966 the percentage of the annual discharge contributed by the spring freshet for the Moisie River has ranged from only 31 to 44% ($\bar{x}$ = 37.7%, SE = 1.2%); in 1980 the percentage was 43%.

What is the long-term pattern? Of the streams chosen, only the Moisie River has a relatively long-term discharge record. Since 1966 the annual discharge pattern has been remarkably consistent with a maximum mean monthly discharge in April–May (1600–1800 $m^3 \cdot s^{-1}$), an occasional secondary autumn peak (600–800 $m^3 \cdot s^{-1}$), and a winter minimum of <150 $m^3 \cdot s^{-1}$ (Naiman 1980; 1981b). The consistent long-term pattern suggests that steady state evaluation of export data is straightforward. Thus, annual export of sediments and carbon is largely influenced by climate which periodically releases most water during spring months, rather than at irregular intervals.

Mean annual discharge can be estimated from basin area. Data from 15 rivers with gauging stations along the North Shore of the Gulf of St. Lawrence demonstrate a highly significant (r = 0.99, $P < 0.01$; t-test) linear relationship between discharge and basin area. Using the equation: log (MAD) = 0.87 log (BA) − 1.17, where, MAD = mean annual discharge ($m^3 \cdot s^{-1}$), and BA = basin area (km^2), it is possible to predict the annual water export. When combined with concentration data the yearly movement of sediments and carbon can be determined for any size watershed in this region.

SEDIMENT AND CARBON CONCENTRATIONS

Moisie concentration matrix — Weighting of particulate loads by position in the stream channel of the Moisie River was not warranted because the water was reasonably well mixed throughout the channel. A significant nonuniform vertical distribution of fine particles in large rivers, as reported by Curtis et al. (1979), can significantly affect estimates of the transport load. For nearly the entire ice-free season no large differences in the percentage organic matter, sediment concentration, or concentration of POM could be detected by position or depth in the Moisie River (Naiman 1982). For any 1 mo the percentage organic matter usually had a range of <20%; differences in sediment concentration were <2.5 $mg \cdot L^{-1}$, and differences in POM concentrations were <0.4 mg $AFDW \cdot L^{-1}$. Although there were some definite trends in concentrations by depth and position between May and July, they were slight (Naiman 1982).

Annual patterns of sediment, POM, and DOC concentrations — All sites have a similar annual pattern of sediment concentration, although peak concentrations vary from stream to stream (Fig. 3). With the onset of ice breakup in April, concentrations at all sites increase and generally remain elevated throughout the spring freshet period. In June, as discharge decreases, suspended sediment concentrations approach low and nearly constant values, remaining at this level

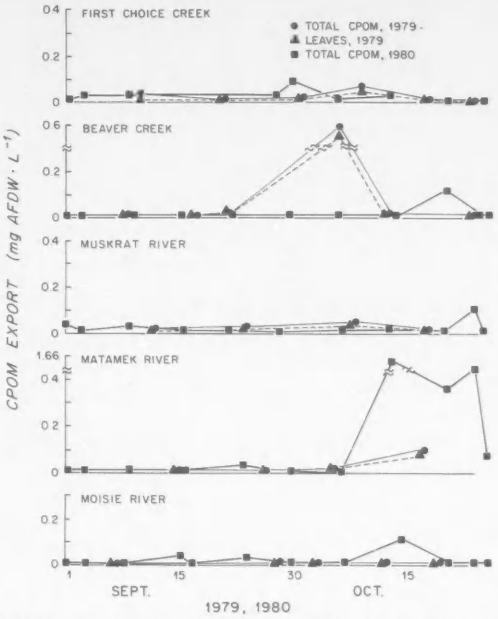

FIG. 6. Concentrations of suspended CPOM are low in all streams; however, during autumn leaf fall the concentration can occasionally increase. Compared to FPOM and DOC, the amount of CPOM exported is negligible.

until the following spring, except for short periods corresponding to intense storms.

Sediment concentrations reported here for non-freshet conditions are comparable to values reported for relatively undisturbed forested streams in mountainous regions (Gurtz et al. 1980; Bormann et al. 1969, 1974), but are considerably less than concentrations in tropical rivers (Richey et al. 1980; Curtis et al. 1979). During freshet conditions, concentrations approach those found in tropical rivers, especially for the Moisie River, but not those in flooding desert streams (Fisher and Minckley 1978). Nevertheless, peak sediment concentrations are less than those found in many disturbed streams elsewhere (Burton et al. 1977; Pierce and Dulong 1977).

For comparison, I plotted POM concentrations on the same scale as sediment concentration (Fig. 4). The most striking features of the annual pattern are that POM concentrations do not increase during the ice breakup/freshet period, concentrations are similar in all streams, and concentrations for a particular site are constant throughout the year.

Mean annual POM concentrations for all sites are comparatively low, ranging from 1.29 mg AFDW·L^{-1} (Moisie River) to 1.85 mg AFDW·L^{-1} (Muskrat River). These values are comparable to those reported for a swamp–stream in North Carolina (Mulholland 1981), and mountain streams in the western and northeastern United States and Canada (Naiman and Sibert 1978; Hobbie and Likens 1973; Naiman and Sedell 1979a; Sedell et al. 1978). In contrast, concentrations in tropical streams are severalfold greater (Brinson 1976; Richey et al. 1980).

During the ice breakup/freshet period DOC concentrations are relatively constant, but slightly less than those measured during the warmer months (Fig. 5). There are differences in DOC concentrations from site to site, but they depend on the characteristics of the watershed rather than a predictable upstream–downstream profile. First Choice Creek and the Moisie River have the lowest concentrations (annual mean = 4.1 and 6.8 mg C·L^{-1}, respectively), while other sites are similar with annual averages of 11.3–12.9 mg C·L^{-1} (Table 1). Higher levels during summer and autumn are possibly due to increased autotrophic production and leaching from newly formed detritus (Naiman 1983).

Dissolved organic carbon concentrations in First Choice Creek and the Moisie River are comparable to blackwater regions elsewhere, while values measured for the other sites are somewhat higher. In Michigan, Wetzel and Manny (1977) found DOC concentrations generally between 3 and 9 mg C·L^{-1}; Richey et al. (1980) measured concentrations in Amazonian blackwaters up to 10 mg C·L^{-1}, whereas Mulholland (1981) found concentrations generally between 10 and 22 mg C·L^{-1} in North Carolina.

Visually, the downstream movement of CPOM seems important only during autumn leaf-fall when an apparently large biomass of alder, paper birch, and trembling aspen leaves are seen. Results indicate, however, that CPOM export is miniscule (<0.1 mg AFDW·L^{-1}), except for brief periods when CPOM concentrations exceed 0.3 mg AFDW·L^{-1}, as seen for Beaver Creek and the Matamek River (Fig. 6). Notably, the high concentrations in October are not observed each year. Apparently all streams are retentive of CPOM. For budget calculations of total carbon export, CPOM can be largely ignored with the possible exception of rare but intense storms when VPOM and FPOM export would be increasing also.

Diel variations — From inspection of Table 2 it appears there is considerable diel variation in concentrations for some dates and sites (however, see below). Usually the largest coefficients of variation are associated with small mean concentrations.

For the single large water mass collected in April 1980, the mean sediment concentration was 0.73 mg·L^{-1} with a coefficient of variation of 65%; the mean POM concentration was 1.05 mg AFDW·L^{-1} with a coefficient of variation of 27%. A separate but similar experiment with DOC resulted in a coefficient of variation of 1%. In many instances these results are comparable to coefficients of variation reported in Table 2, indicating that diel changes in sediment, POM, and DOC are slight, and in all cases natural variations are probably <10%.

Annual pattern of DOC:POC ratio — During the ice breakup/freshet period the DOC:POC ratio is low (usually <10) and nearly constant in First Choice Creek and the Moisie River (Fig. 7). With decreasing discharge in June the ratio increases significantly, approaching 40, before decreasing again. The other sites generally have ratios higher and more variable than First Choice Creek or the Moisie River. All streams have high DOC:POC ratios in June and July, lower ratios in August, and high ratios again in autumn. Reasons for this pattern are not clear.

TABLE 2. The mean concentration and coefficient of variation of diel sample series at each site are given by date for sediment, POM, and DOC concentrations.

Site/date	Sediment ($mg \cdot L^{-1}$) $\bar{x}$	Sediment CV(%)	POM (mg AFDW $\cdot L^{-1}$) $\bar{x}$	POM CV(%)	DOC (mg $C \cdot L^{-1}$) $\bar{x}$	DOC CV(%)
First Choice Creek						
May 5–6 80	8.83	48	2.05	38	3.02	9
July 5–6 80	1.38	52	1.38	87	5.23	58
July 29–31 80	1.67	31	0.99	23	2.98	33
Sept. 4–5 80	3.12	47	1.53	30	3.02	26
Beaver Creek						
May 5–6 80	0.78	65	1.41	23	9.52	4
July 5–6 80	1.76	102	1.53	45	13.93	9
July 29–31 80	0.56	24	2.07	10	14.11	9
Sept. 4–5 80	1.53	283	2.49	41	—	—
Muskrat River						
May 5–6 80	3.37	39	1.73	10	11.54	6
July 5–6 80	0.03	500	1.56	12	10.04	12
July 29–31 80	0.57	99	1.86	12	9.33	9
Sept. 4–5 80	0.03	—	3.09	12	18.82	18
Matamek River						
May 5–6 80	2.81	14	1.63	13	10.27	4
July 5–6 80	0.48	56	0.97	12	10.11	13
July 29–31 80	0.36	157	1.46	14	7.88	4
Sept. 4–5 80	0.81	70	1.97	11	14.38	12
Moisie River						
May 5–6 80	27.41	65	4.78	60	7.86	2
July 5–6 80	0.77	62	0.73	37	7.22	27
July 29–31 80	0.66	35	0.68	34	5.27	17
Sept. 4–5 80	1.47	42	0.83	17	5.48	7

The DOC:POC ratio is usually <10 only during the spring freshet, which contrasts with values reported for many other streams. Wetzel and Manny (1977), Naiman and Sedell (1979a), Brinson (1976), and others found DOC:POC ratios to be generally <10 throughout the year, although Mulholland (1981) found ratios in swamp streams comparable to those in Quebec.

Annual pattern of percentage organic matter — There is considerable annual variation in the percentage of organic matter in the suspended load (Fig. 8). First Choice Creek and the Moisie River export particulate seston which tends to be only about 50% organic matter. At other sites particulate seston is about 50–75% organic matter only during the ice breakup/freshet; at other times it is usually >80%. This can be important for biotic communities, especially filter-feeding organisms, which require a constant source of organically rich particles (Wallace et al. 1977).

The relatively low percentages of organic matter during the spring freshet are indicative of increased erosion and transport of terrigenous material. Bormann et al. (1974) found the percentage of organic matter in small forest streams ranged from 27 to 82% ($\bar{x}$ = 48%), whereas in a recently clear-cut watershed the percentage ranged from 11 to 82% ($\bar{x}$ = 35%) with the percentage of organic matter decreasing in the cut area over the 4-yr study. Likewise, Pierce and Dulong (1977) found the percentage of organic matter generally <40% and widely variable during a 2-yr study of the Rhode River, Maryland. Naiman and Sedell (1979a) showed the percentage of organic matter in Oregon streams varying seasonally with stream order and particle-size grouping. In the latter study, small streams (1st and 3rd order) exported material with organic matter concentrations generally >60% while the larger streams (5th and 7th order) seasonally exported material with organic matter percentages <40%. This indicates that either erosional processes increase downstream or more sediment is available, at least through 7th-order streams, or that there is a selective retention and use by the biota of organic particles in upstream reaches.

Particle-size analysis — In the smaller streams, especially First Choice Creek, there is a higher proportion by weight of large particles transported than in the rivers (Fig. 9). In First Choice Creek 45% of the particulate carbon transported is associated with material >53 μm diameter. Once moved downstream particle-size reduction proceeds rapidly. In Beaver Creek only about 8–10% of the transported weight is associated with particles >53 μm diameter; in larger streams it is <5%. Considering that nearly all organic inputs to First Choice Creek are in the form of wood and leaves (Conners and Naiman 1982), the amount of material reduced to <1 mm before export is remarkable, presenting strong evidence for the completeness of organic matter processing in headwater systems. The fact that particle-size reduction is so advanced by the time suspended particles reach 3rd-order streams further strengthens this argument.

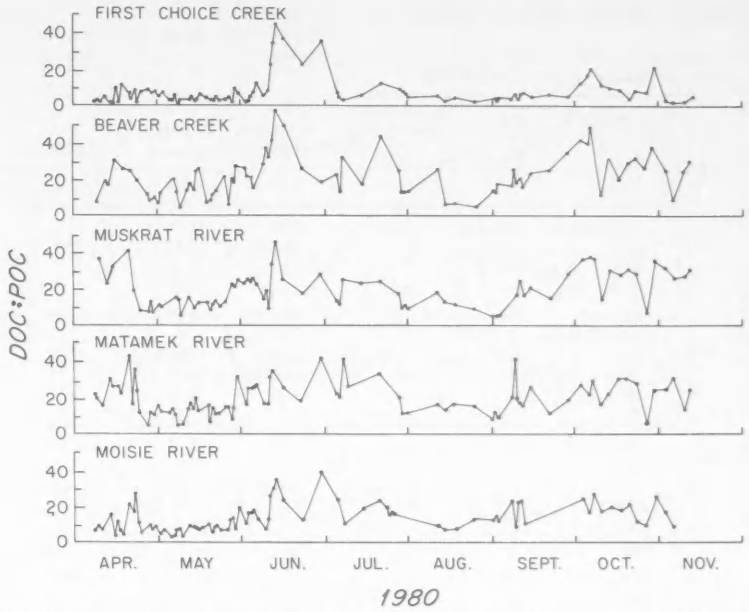

FIG. 7. The annual pattern of the DOC:POC ratio varies on a daily basis for most streams. Ratios tend to be greatest during summer and autumn and lowest during spring freshet.

These data are mostly in accordance with results presented by Sedell et al. (1978) and Naiman and Sedell (1979a) on organic particle sizes in streams throughout North America. In more extensive analyses they found that mean particle size does not change among stream orders or biomes, is not affected by discharge regimes, and remains between 3 and 12 μm diameter. Thus, they argued that processing of allochthonous inputs is highly efficient in undisturbed 1st-order streams and, in addition, that these headwater streams are retentive of organic matter because of a large number of devices holding this material in place (see also Bilby and Likens 1980).

CONCENTRATIONS AS A FUNCTION OF HYDROLOGY

Correlations — Only in a few instances did concentrations or ratios regress reliably against discharge or unit stream power (Table 3), suggesting that availability of materials may be more important than erodibility. Although most correlations are significant because of the large sample size, in nearly all cases discharge explains <30% of the variation in concentrations or ratios, and unit stream power explains <20%. Notable exceptions are the Muskrat River where hydrology accounts for 40% of the variability in the percentage of organic matter and 69% of the variability in sediment concentrations. In the Moisie River 34% of sediment, 36% of POM concentrations, and 32% of the variability in DOC concentrations are explained by hydrology.

Relationships are not improved by combining data from all sites (Table 3, Fig. 10). Discharge explains only 40% of the variation in percentage organic matter, 23% of the variation in sediment concentration, and 24% of the variation in DOC concentration. Discharge and unit stream power have no effect on POM concentrations; in both cases they explain <3% of the variation.

Correlations between discharge or stream power and the suspended sediment load have been previously described (Leopold et al. 1964; Morisawa 1968; Dunne and Leopold 1978). Geomorphologists tend to treat a river as a transporting machine that, like other machines, can be characterized by the relation: rate of doing work = available power × efficiency. If true, then correlations between discharge or stream power and material concentrations would be straightforward whereas, in fact, they are not. For watersheds studied here, and probably elsewhere, stream power and discharge are not especially good predictors of sediment and organic matter concentrations (Bormann et al. 1974; Sedell et al. 1978; Naiman and Sedell 1979a; Bilby and Likens 1979). This suggests that retention devices in streams are more efficient than previously thought and may actually have the key role in determining suspended concentrations. This is especially evident when one considers that only a small percentage (e.g. most cases <10%) of variability in concentrations can be attributed to discharge or stream power.

The important role of retention devices (see also p. 1713) becomes even more evident when it is considered that POM, because of a low specific weight relative to inorganic sediment, should respond faster to increases in discharge than do sediments. Nevertheless, POM shows virtually no response to discharge or stream power, while the relatively heavier sediment demonstrates some positive response to variations in

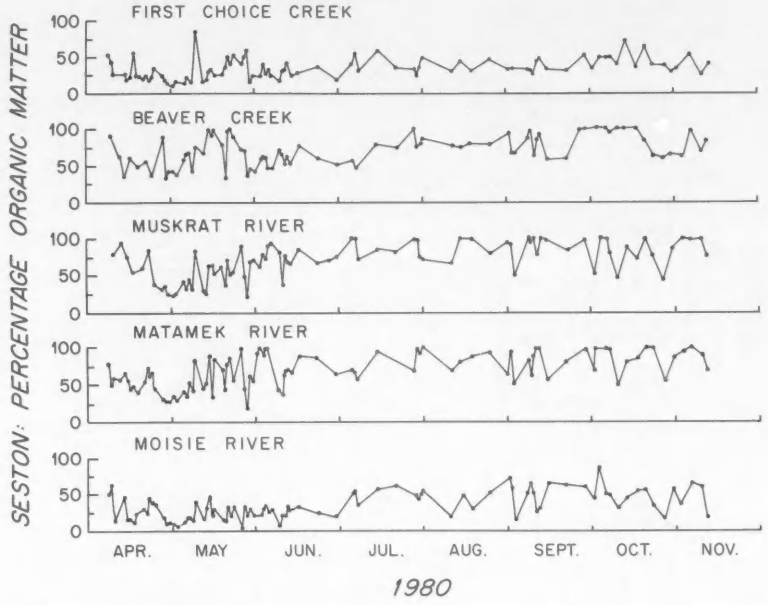

FIG. 8. The percentage of particulate organic matter in the seston fluctuates considerably from day to day at all sites, thus influencing the apparent food quality of drifting material.

discharge. Therefore, within the watershed, retention devices are more efficient in retaining POM and DOC than sediment, which is in contrast to expectations based on the physical properties of particles and discharge alone. More experimental studies similar to those of Bilby (1981) and Bilby and Likens (1980) are needed to assess properly the relative importance of retention devices.

Summer storms — The effects of brief but intense storms on transport of fine particulate organic carbon in small streams recently has been addressed by Bilby and Likens (1979) and Gurtz et al. (1980). These studies revealed maximum concentrations of POM occurring as a pulse before peak discharge is reached. They suggest that rainfall itself, rather than increasing flow, appears responsible for this early pulse. These concepts are important for an accurate description transport phenomenon but the applicability to larger streams and rivers has not been demonstrated. Hydrologically, the response of rivers to storms is fundamentally different from that of small streams; hence, it is informative to examine a storm series in detail for the five streams simultaneously.

Betwen August 3 and 8, 1980, a series of intense rain showers left a total accumulation of 3.58 cm — a substantial accumulation for this month. The preceeding 2 weeks had been dry and clear. Late on the evening of August 3 there was a series of brief but mild thunderstorms; on August 4 rain and fog persisted throughout the day; August 5 was cloudy but dry; on August 6 severe thunderstorms occurred in the evening; August 7 experienced intermittent drizzle; and on August 8 there was a very brief rain shower. The response of

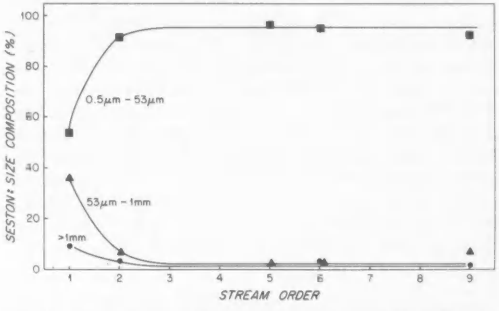

FIG. 9. The size composition of suspended particulate organic matter shifts quickly to very small particles as stream size increases. Over 90% of drifting POM in streams larger than 2nd order is <53 μm diameter.

each stream was different (Fig. 11). Only the Muskrat and Matamek River discharges responded to the rain on August 4 with rising waters delayed until August 5. All streams, except the Moisie River, reacted immediately to the severe thunderstorms on August 6. The discharge in the Moisie River rose gradually over the following days.

The response of sediment, POM, and DOC concentrations depended upon the site and storm (Fig. 11). No increase in concentrations in response to the rain on August 4 occurred for any stream, despite discharge in the Muskrat River nearly doubling in a 3-h period and the Matamek River discharge

TABLE 3. Correlations are given between the concentrations or ratios of suspended material being carried by each stream and discharge or unit stream power.

			Discharge		Stream power	
Stream	Parameter	Sample size (n)	Coefficient of determination (r^2)	Significance (p)	Coefficient of determination (r^2)	Significance (p)
First Choice Creek	%OM	172	0.03	0.05	0.03	0.05
	SED	172	0.01	ns	0.01	ns
	POM	172	0.07	0.01	0.07	0.01
	DOC	168	0.14	0.01	0.13	0.01
	S:P	172	0.11	0.01	0.11	0.01
	D:P	168	0.00	ns	0.00	ns
Beaver Creek	%OM	164	0.03	0.05	0.06	0.05
	SED	164	0.08	0.01	0.03	0.05
	POM	164	0.03	0.05	0.02	ns
	DOC	146	0.06	0.01	0.02	ns
	S:P	164	0.04	0.05	0.04	0.05
	D:P	146	0.04	0.05	0.04	0.05
Muskrat River	%OM	164	0.40	0.01	0.39	0.01
	SED	164	0.69	0.01	0.69	0.01
	POM	164	0.14	0.01	0.14	0.01
	DOC	144	0.10	0.01	0.10	0.01
	S:P	164	0.53	0.01	0.54	0.01
	D:P	144	0.05	0.05	0.05	0.05
Matamek River	%OM	172	0.14	0.01	0.14	0.01
	SED	172	0.15	0.01	0.15	0.01
	POM	172	0.09	0.01	0.09	0.01
	DOC	165	0.01	ns	0.01	ns
	S:P	172	0.12	0.01	0.13	0.01
	D:P	165	0.05	0.05	0.05	0.05
Moisie River	%OM	170	0.18	0.01	0.18	0.01
	SED	170	0.34	0.01	0.33	0.01
	POM	170	0.36	0.01	0.36	0.01
	DOC	161	0.32	0.01	0.32	0.01
	S:P	170	0.19	0.01	0.20	0.01
	D:P	161	0.14	0.01	0.07	0.01
All streams	%OM	842	0.40	0.01	0.02	0.01
	SED	842	0.23	0.01	0.01	0.05
	POM	842	0.02	0.01	0.03	0.01
	DOC	784	0.29	0.01	0.03	0.01
	S:P	842	0.32	0.01	0.02	0.01
	D:P	784	0.12	0.01	0.05	0.01

increasing by almost 20%. For the thunderstorm on August 6 only First Choice Creek and Beaver Creek increased sediment and POM concentrations preceeding the rise in discharge, whereas DOC concentrations rose with discharge. In the rivers no corresponding increase in particulate or dissolved concentrations was observed, except possibly for a slight rise in DOC concentration in the Moisie River. Thus, it appears the response of rivers to summer storms is minimal, and for small streams the response is short-lived as reported by Bilby and Likens (1979).

RATING CURVES

Daily suspended load ($kg \cdot d^{-1}$) is usually a linear function of discharge, and the resulting relationship permits development of a rating curve for each export parameter (Leopold et al. 1964). This allows prediction, within limits of the curve, of loads carried by a given discharge and becomes useful in estimating annual loads for rivers where only the discharge regime is measured regularly.

Rating curves for sediment, POM, and DOC were constructed using data from 1979 to 1981 for all sites combined (Naiman 1982). Between discharges of 0.001 $m^3 \cdot s^{-1}$ and 2000 $m^3 \cdot s^{-1}$ it is possible to estimate daily yield reliably. The weakest relationship is for sediment where $r^2 = 0.80$ [log(SED) = 1.84 + 0.97 log(D), $n = 828$]; whereas, for POM [$r^2 = 0.98$; log(POM) = 2.07 + 0.98 log(D); $n = 828$] and DOC [$r^2 = 0.98$; log(DOC) = 2.79 + 1.05 log(D); $n = 770$] the relationships are strong. Data for each stream follow a similar pattern but site-specific correlations were weaker, especially for sediment in First Choice Creek ($r^2 = 0.17$) and the Matamek River ($r^2 = 0.20$).

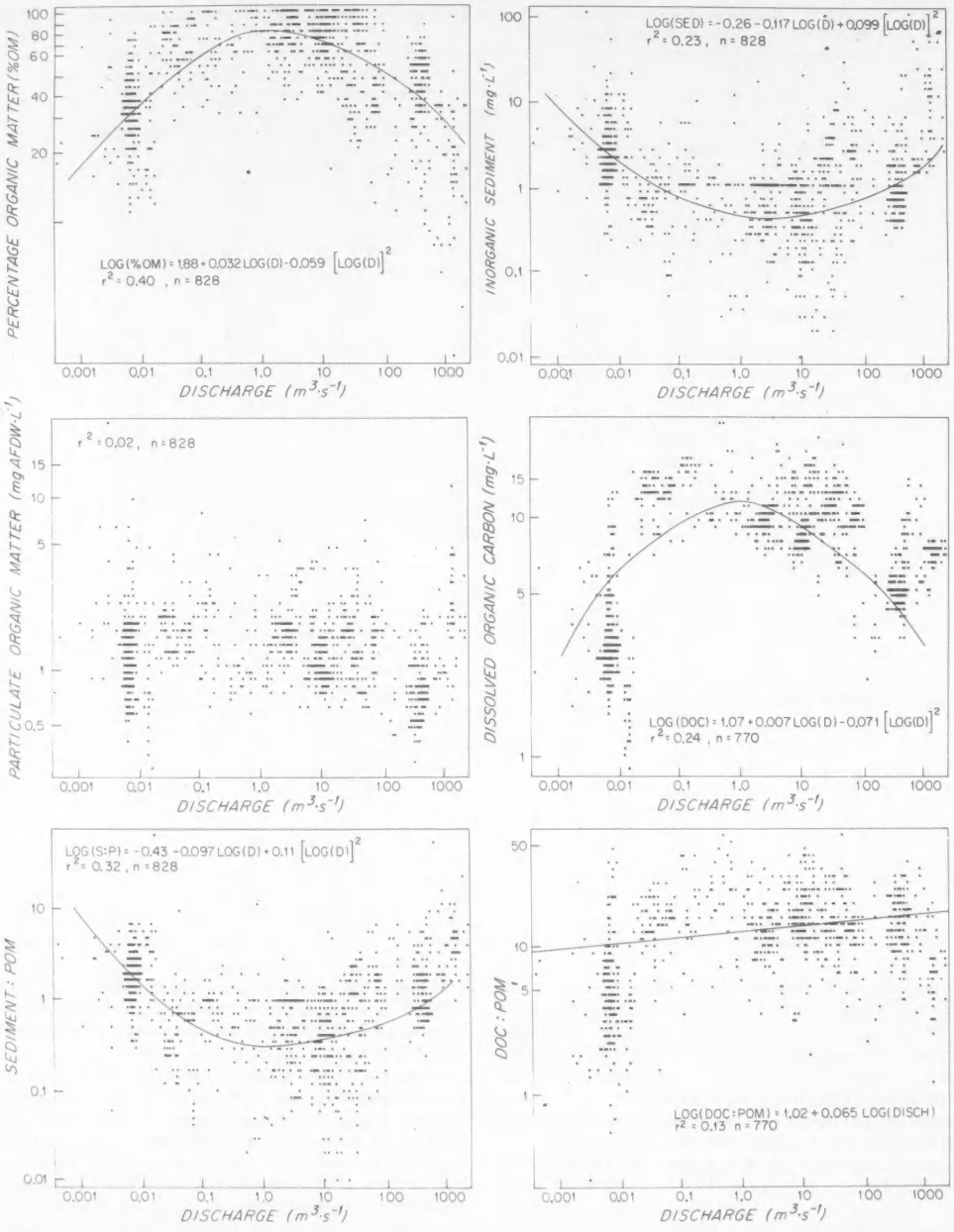
PERCENTAGE ORGANIC MATTER (%OM)
LOG(%OM) = 1.88 + 0.032 LOG(D) - 0.059 [LOG(D)]²
r² = 0.40 , n = 828
DISCHARGE (m³·s⁻¹)
INORGANIC SEDIMENT (mg·L⁻¹)
LOG(SED) = -0.26 - 0.117 LOG(D) + 0.099 [LOG(D)]²
r² = 0.23 , n = 828
DISCHARGE (m³·s⁻¹)
PARTICULATE ORGANIC MATTER (mg AFDW·L⁻¹)
r² = 0.02 , n = 828
DISCHARGE (m³·s⁻¹)
DISSOLVED ORGANIC CARBON (mg·L⁻¹)
LOG(DOC) = 1.07 + 0.007 LOG(D) - 0.071 [LOG(D)]²
r² = 0.24 , n = 770
DISCHARGE (m³·s⁻¹)
SEDIMENT : POM
LOG(S:P) = -0.43 - 0.097 LOG(D) + 0.11 [LOG(D)]²
r² = 0.32 , n = 828
DISCHARGE (m³·s⁻¹)
DOC : POM
LOG(DOC:POM) = 1.02 + 0.065 LOG(DISCH)
r² = 0.13 n = 770
DISCHARGE (m³·s⁻¹)

FIG. 10. Data from all sites for percentage organic matter, sediment, POM, DOC, sediment : POM ratio, and DOC : POM ratio are combined and regressed against discharge. The resulting relationships are weak with <40% of the variation in each case attributable to discharge.

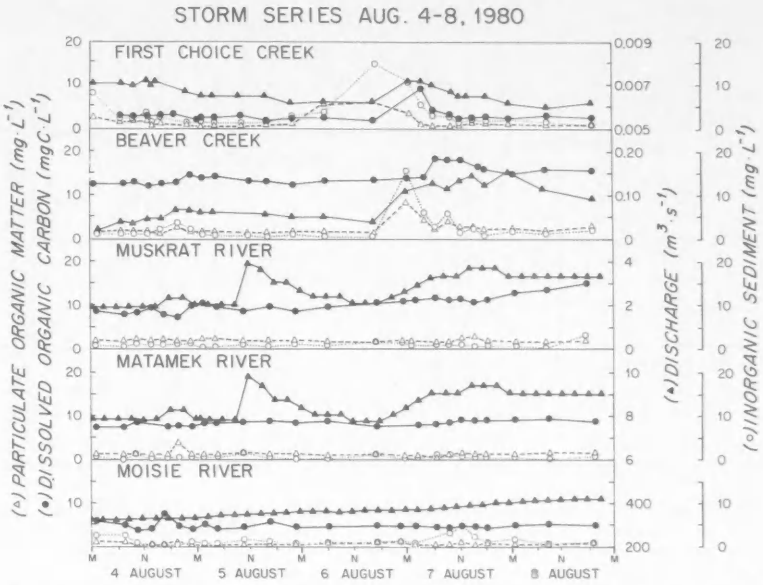

FIG. 11. The effect of summer storms on the hydrologic regime, and sediment, POM, and DOC concentrations is shown for each site. During this period 3.58 cm of rain fell in two storms.

Correlations between the daily loads of POM, DOC, and discharge remained strong ($r^2 > 0.8$ in most cases).

These relationships allow estimation of export loads for previous years. Discharge records for the Moisie River are complete for the years 1966 and 1968–78. During this period the estimated annual export of sediment varied between 4.1×10^7 and 7.9×10^7 kg·yr^{-1} ($\bar{x} = 6.36 \times 10^7$; SE $= 0.32 \times 10^7$); the annual export of POM was between 1.5×10^7 and 1.9×10^7 kg AFDW·yr^{-1} ($\bar{x} = 1.75 \times 10^7$; SE $= 0.04 \times 10^7$); and for DOC it was between 1.3×10^8 and 1.6×10^8 kg C·yr^{-1} ($\bar{x} = 1.46 \times 10^8$; SE $= 0.03 \times 10^8$).

Synthesis

Retention Devices

The natural stream channel is a complex array of physical structures, chemical reactions, and biotic processes acting to influence the movement of materials downstream. Mechanisms by which specific components of this array act to influence the character of seston are still largely speculative but, considering results reported here, are deserving of some discussion.

The nature of retention devices is not constant along the river continuum, rather there are apparent differences between low-, middle-, and high-order streams. For example, physical structures controlling the routing of materials in low-order streams are largely debris jams which trap materials and create the characteristic stair-step channel morphology of small streams (Swanson and Lienkaemper 1978; Bilby 1981). Larger rivers often have waterfalls separated by long reaches of low gradient. A considerable amount of potential energy is dissipated at falls, while material can be stored in the low-gradient reaches. Additionally, in higher-order streams, alcoves and eddies are formed as the channel develops a typical meandering pattern. In alcoves and eddies lighter organic matter is physically sorted from heavier sediment by the characteristic water movement. Corresponding with a decrease in the water's energy, heavier inorganic sediments are deposited nearer the main stream channel as sandbars, while fine organic matter is deposited in backwaters or alcoves as the eddy diffuses (for example, see Dietrich et al. 1979). Large rivers also have well-developed flood plains where considerable POM and fine sediment are deposited during periods of high flow.

This sorting may be a key factor in explaining why sediments respond more closely to discharge than POM, that is, the availability of each particle type is different. Sediment is readily available in sandbars and river banks while organic matter is either associated with the terrestrial watershed or retained and concentrated by eddies in backwater areas. This process of physical separation would be operating continuously, even during rising water.

Chemical processes affecting retention of organic matter are less well known. Nevertheless, it has been recently shown that this pathway may be significant as dissolved carbon and nutrients are effectively retained by flocculation or microbial uptake (Lock and Hynes 1975; Lock et al. 1977; Newbold et al. 1981). Carbon and nutrients are passively carried by the water in their dissolved form, but when converted to particulate matter they can be selectively retained in the watershed by

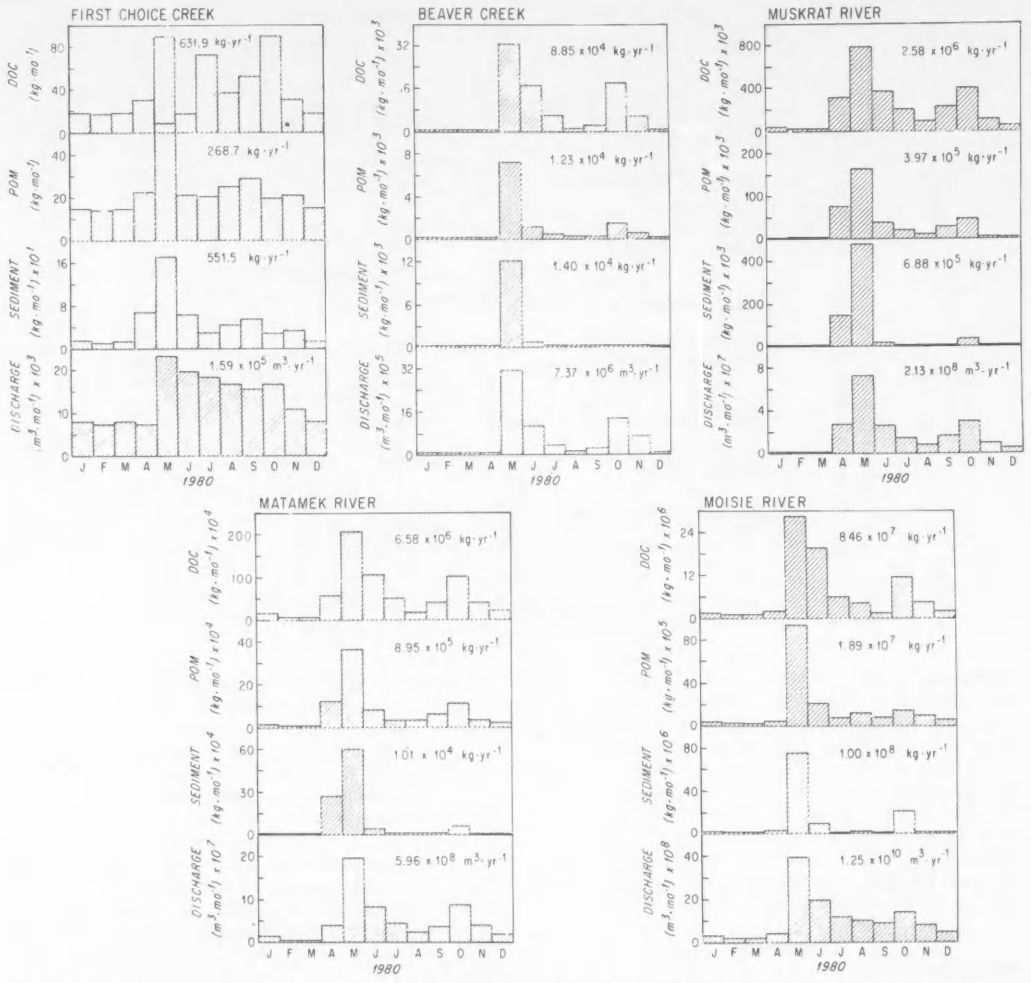

FIG. 12. The monthly pattern of total sediment, POM, and DOC export for 1980 is given for each site. Most sediment is exported during spring freshet; whereas total POM and DOC export follow the annual hydrograph.

eddies or surface attachment, and eventually consumed by invertebrates.

A final consideration is that POM is continually subjected to biotic oxidation by microorganisms and invertebrates, while sediment remains largely intact. The role of organisms, especially the filter feeders, in removing organic matter from the water column has been well documented (Wallace et al. 1977; Wallace and Merritt 1980). These organisms are efficient in capturing organic materials, serving to retain POM effectively in the watershed where it is oxidized to CO_2. McCullough et al. (1979) calculated that filter feeders in a 1-m^2 area of Deep Creek, Idaho, were able to remove 0.01% of the seston flowing over them each day. At this rate they calculated that complete removal of suspended organic matter by filter feeders would occur in only 9 km.

TABLE 4. Percentage of the total annual export occurring during the spring freshet, April 15 to June 14, 1980.

Stream	Discharge	Sediment	POM	DOC
First Choice Creek	24.0	60.6	31.2	26.9
Beaver Creek	55.0	90.3	65.4	51.4
Muskrat River	52.0	91.9	64.6	48.1
Matamek River	48.0	89.7	59.2	49.2
Moisie River	43.0	70.6	58.5	47.3

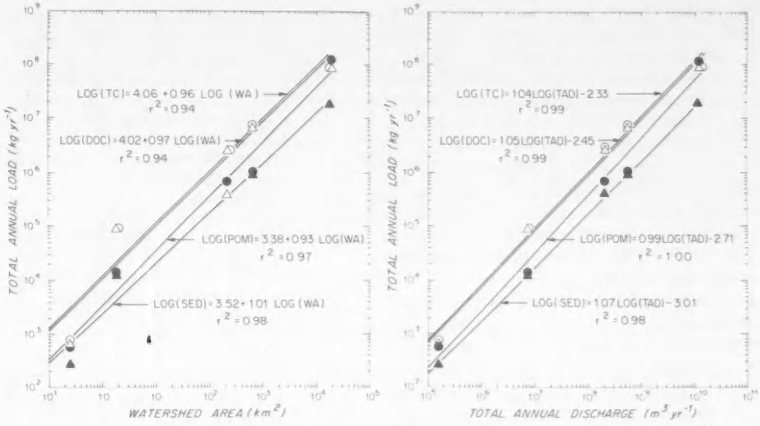

FIG. 13. Total annual export of sediment, POM, DOC, and TOC can be reliably predicted from either watershed area or total annual discharge. In both cases all coefficients of determination (r^2) are >0.94.

TABLE 5. Annual yield of sediment and carbon per unit area for the five watersheds in 1980. Total carbon is calculated by assuming POM to be 50% carbon.

Stream	Watershed area (km^2)	Sediment ($g \cdot m^{-2} \cdot yr^{-1}$)	POM (g AFDW $\cdot m^{-2} \cdot yr^{-1}$)	DOC (g C $\cdot m^{-2} \cdot yr^{-1}$)	Total carbon (g C $\cdot m^{-2} \cdot yr^{-1}$)
First Choice Creek	0.25	2.21	1.08	2.53	3.07
Beaver Creek	1.83	7.62	6.73	48.38	51.75
Muskrat River	207	3.32	1.92	12.44	13.40
Matamek River	673	1.50	1.33	9.78	10.45
Moisie River	19871	5.99	0.95	4.26	4.74

ANNUAL EXPORT PATTERN

Based on observations of hydrological patterns and the available literature, it was expected organic matter and sediments would accumulate in each stream during the low-discharge, low-power period lasting from late autumn to ice breakup in April. As discharge rapidly increased in spring it was anticipated that most fine organic matter and sediments would be scoured downstream, and some larger particles (e.g. trees, cobbles, boulders) would be redistributed. This mass movement would be manifested by increased concentrations of DOC, POM, and suspended sediment for a short period at the start of spring runoff. This scenario is consistent with observations made by Bilby and Likens (1979), Bormann et al. (1969, 1974), Hobbie and Likens (1973), and others, who have examined export from small watersheds. The major differences between previous studies and this one are that the former watersheds had at one time received a severe perturbation (e.g. logging or road building), and that all of those watersheds were small (<0.2 km^2).

The bulk export of organic matter mimics, to a large extent, the annual discharge pattern of each stream (Fig. 12). However, most sediment export occurs during spring freshet. The total amount of material leaving each watershed is, of course, dependent upon basin size, i.e. water discharge. The majority of organic matter is in dissolved form; this fraction is usually an order of magnitude greater than particulate export. For most of these streams, annual sediment yield is 50% greater than POM export. For sediment, POM, and DOC the annual pattern is similar between streams, regardless of watershed size.

The effect of the spring freshet on bulk sediment and carbon export reveals fundamentally different annual patterns among materials (Table 4). First Choice Creek exports 61% of the annual sediment yield but 31% of the annual POM load and 27% of the annual DOC output (Table 4) during the spring freshet (i.e. 24% of the annual discharge between April 15 and June 14). The other sites, where 43–55% of the annual discharge occurs during the same period, have a similar pattern. Between 71 and 92% of the annual sediment load is carried during freshet while 59–65% of the annual POM yield and 47–51% of the annual DOC output occurs in the same period. The spring freshet exerts considerable influence on the movement of sediment, while carbon export is more conservative. This is surprising for POM as it has a lower specific gravity than sediment, and would be expected to be the first particles transported. This suggests that in-stream POM retention devices are strong and influence particle movement either through a capacity to resist the water's physical force or by ability to retain POM selectively. An alternative hypothesis is that the standing stock of POM is relatively low at the start of freshet after a long winter of processing, and the small amount available is then exported over a short period.

The annual yield of sediment and organic matter can be reliably estimated from either watershed area or total annual discharge (Fig. 13). For sediment, coefficients of determination (r^2) are >0.94 whereas for organic matter all coefficients are >0.98. Equations describing the respective relationships are not fundamentally different from those calculated by Schlesinger and Melack (1981) for a variety of the world's rivers. They caution that none of the rivers used in their calculations were carefully studied during all stages of river flow comprising a hydrologic year, and there were no data for the sediment-rich rivers of Asia. The agreement of data reported here for a complete hydrologic cycle lend support to relationships they reported.

Another approximation of sediment and carbon yield is based on the denudation rate of terrestrial ecosystems. Losses of organic carbon are based on samples taken at downstream sites; therefore, calculated denudation rates are minimum estimates as carbon oxidized during transport is not included. Sediment yield is roughly similar for all sites ranging from 1.5 $g \cdot m^{-2} \cdot yr^{-1}$ in the Matamek River to 7.6 $g \cdot m^{-2} \cdot yr^{-1}$ in Beaver Creek (Table 5). Annual POM export is nearly identical in all streams (~1 g $AFDW \cdot m^{-2} \cdot yr^{-1}$), except for Beaver Creek (6.7 g $AFDW \cdot m^{-2} \cdot yr^{-1}$). The amount of dissolved carbon exported is 4–14 times larger than annual POC export, ranging from 2.5 g $C \cdot m^{-2} \cdot yr^{-1}$ in First Choice Creek to 48.4 g $C \cdot m^{-2} \cdot yr^{-1}$ in Beaver Creek. Total organic carbon export ranges from 3.1 g $C \cdot m^{-2} \cdot yr^{-1}$ in First Choice Creek to 51.8 g $C \cdot m^{-2} \cdot yr^{-1}$ in Beaver Creek. Other than Beaver Creek, all sites export <14 g $C \cdot m^{-2} \cdot yr^{-1}$. The high denudation rates for Beaver Creek may be due to the extensive beaver activity which influences DOC export by increasing the water surface area in contact with organic sediments (see Mulholland and Kuenzler 1979) and increases POM export by adding massive amounts of woody debris to the stream channel.

Vannote et al. (1980) theorized that the seasonally pulsed nature of organic inputs is damped by biological processes and retention, such that total export of organic matter does not increase downstream for undisturbed river systems. From data reported here the yield of material per unit of watershed area appears to decrease downstream, again emphasizing the importance of instream processing and retention.

The denudation rates for carbon, except for Beaver Creek, compare favorably to rates reported by Schlesinger and Melack (1981) for wetlands and some large boreal forest watersheds. First Choice Creek and the Moisie River compare well to denudation rates reported for rivers in northeast Canada and New England by Mulholland and Watts (1982). However, the Muskrat River, Matamek River, and Beaver Creek do not fall within the range they reported, but tend to confirm that TOC export is high in northeastern North America.

Oxidation rates — The yield of carbon from a watershed via the drainage system is the sum of material passing a point plus the amount of material oxidized during transport. Weight specific respiration rates of drifting VPOM, FPOM, and CPOM were estimated (Naiman 1980) and will be formally reported elsewhere. Assuming all respiration is associated with VPOM and FPOM in First Choice Creek (see Fig. 9 for particle-size distribution) and only VPOM at the other sites, it is possible to approximate the annual oxidation pattern and estimate the total amount of carbon oxidized in transit (Fig. 14). The seasonal pattern for total carbon oxidized is slightly different for each stream. Maximum rates for two sites occur in summer (First Choice Creek, Moisie River), one during the spring freshet (Beaver Creek), one in autumn (Matamek River), and one in both spring and autumn (Muskrat River). Peak rates for all sites are usually about 1–1.5% $\cdot d^{-1}$. Surprisingly, the annual average for all sites is nearly constant, ranging from only 0.30 to 0.51% $\cdot d^{-1}$. This lends some support to the concept of Vannote et al. (1980) that rate of energy utilization is constant throughout the drainage network in undisturbed river systems.

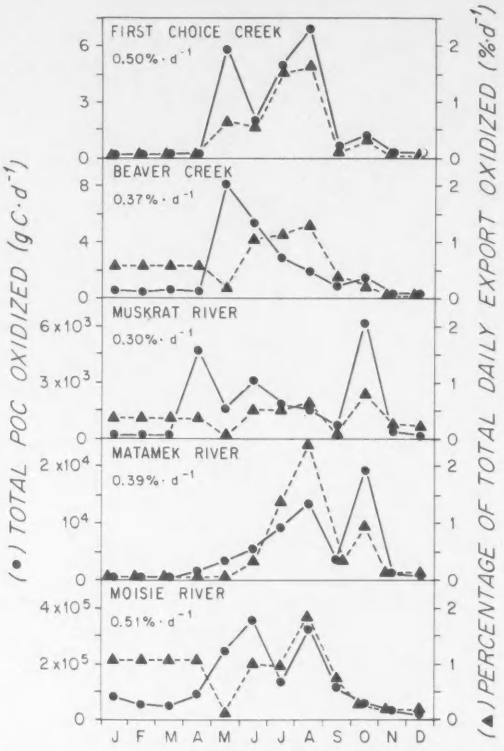
FIRST CHOICE CREEK
0.50% · d⁻¹
BEAVER CREEK
0.37% · d⁻¹
MUSKRAT RIVER
0.30% · d⁻¹
MATAMEK RIVER
0.39% · d⁻¹
MOISIE RIVER
0.51% · d⁻¹
(•) TOTAL POC OXIDIZED (g C · d⁻¹)
(▲) PERCENTAGE OF TOTAL DAILY EXPORT OXIDIZED (% d⁻¹)
J F M A M J J A S O N D

FIG. 14. Percentage of POM oxidized annually remains essentially constant as stream size increases; however, annual oxidation pattern varies according to the specific characteristics of each stream ecosystem.

Oxidation rates of drifting POM have seldom been systematically measured. Richey et al. (1980) report that during rising water in the Amazon River 3% of the POC pool turned over each day during low water and about 0.3% at high water. For a particular discharge level, the utilization rate was constant throughout the river. These results, and those reported by Naiman and Sedell (1979a), are in general agreement with

TABLE 6. Total annual export from all streams of a particular order is compared to the amount of organic matter available in the watershed annually. Total organic input is the sum of the VPOM and FPOM standing crop, litter and lateral inputs >1 mm diameter, and net annual primary production. Geomorphological data are based on the Moisie River watershed; VPOM and FPOM standing crop and net annual primary production data are from Naiman (1980, 1983), and allochthonous input data are from Conners and Naiman (1982).

Stream	Stream order	No. of streams	Total organic input (kg AFDW·yr^{-1})	Total annual POM export (kg AFDW·yr^{-1})	POM export/ total input
First Choice Creek	1	38 770	0.4×10^7	1.0×10^7	2.5
Beaver Creek	2	8 400	1.2×10^7	1.0×10^8	8.3
Muskrat River	5	95	4.6×10^7	2.7×10^7	0.6
Matamek River	6	19	5.1×10^7	1.7×10^7	0.3
Moisie River	9	1	6.6×10^7	1.9×10^7	0.3

the decomposition rate of drifting POM reported here and its constant value along the river continuum.

Annual export vs. available material — The amount of organic matter available annually for export in stream channels above the point of sampling can be approximated by combining previously determined geomorphological data with information on the standing stock of detritus of various stream orders (Naiman 1980, 1983), total allochthonous inputs (Conners and Naiman 1982), and primary production (Naiman 1983). Using data from the Moisie River watershed it is estimated, for example, that there are 38 770 streams similar to First Choice Creek with total of 0.4×10^7 kg AFDW potentially available for export annually (Table 6). These streams, in total, export an estimated 1.1×10^7 kg·yr^{-1} of POM or ~2.5 times more than the estimated available organic matter. Beaver Creek exports 8.3 times more than the estimated amount readily available, while the Muskrat, Matamek, and Moisie rivers export only 0.3–0.6 of available organic matter. Also, some organic material is oxidized by the biota, further supporting the fact that organic inputs have been underestimated.

These data, although admittedly approximations, point out two ecosystem level features. First, there must be a continuous and fairly rapid movement of organic matter from the terrestrial landscape to small streams. This influx may occur either as DOC flocculation (Lock and Hynes 1975; Lock et al. 1977) or during storms as dendritic rivulets develop in otherwise terrestrial habitat, or by wind during drier periods. The magnitude and timing of these latter inputs have not been measured for any stream ecosystem. The second ecosystem feature is that even though small streams and rivers export more POM than the amount calculated to be available annually, most material is either stored within larger rivers or processed by the biotic community. This is supported by the general tendency for the POM export : total input ratio (Table 6) to decrease downstream, and by the low and constant ratio achieved somewhere between 2nd- and 5th-order streams.

Conclusions

Results suggest that in-stream retention devices have a strong influence on organic carbon concentrations in pristine streams, regardless of watershed size, while discharge and stream power have little effect. Both the spring freshet and summer storms have been shown to have little control over organic carbon concentrations. Sediment movements respond to discharge patterns, suggesting that export of sediment and organic material are controlled by two different processes: retention characteristics and the water's potential energy. Annual yields (g·m^{-2}·yr^{-1}) reported here are similar to those reported previously for boreal forests but differ slightly in response to watershed size. The particulate organic material being exported has been efficiently processed, regardless of stream size, as evidenced by the dominance of VPOM, the minor contribution of CPOM, and the fact that only 0.3–0.5% of the POM is oxidized daily during movement. These data emphasize that retention and processing of organic matter within the pristine watershed is an important and complicated phenomenon, and that the downstream movement of this material cannot be easily described just in terms of fluid dynamics. Further studies of an experimental nature will be required to quantify the relative importance of specific in-stream retention devices. These must be completed before a basic understanding of factors controlling sediment and organic carbon concentrations can be fully achieved.

Acknowledgments

I thank S. George, S. Zimmermann, F. G. Whoriskey, and D. LaChance for assistance with the field program and B. Farr for data management and statistical analyses. D. Aubrey, J. Milliman, P. Mulholland, J. Richey, and J. Sloane-Richey made helpful comments and suggestions for improvement. Financial support was provided by the Matamek Research Program.

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NOTES

Use of Box and Line Plots to Assess Fish Ventilatory Behavior in Biological Monitoring Systems

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SYDOR, W. J., W. R. MILLER III, J. CAIRNS JR., AND D. GRUBER. 1982. Use of box and line plots to assess fish ventilatory behavior in biological monitoring systems. Can. J. Fish. Aquat. Sci. 39: 1719 – 1722.

Ventilatory signals from 12 bluegill (*Lepomis macrochirus*) sunfish were collected with the aid of an on-line minicomputer as part of a biological monitoring system. Our results from three experiments demonstrate a potential for applying these graphic techniques to on-line biomonitoring systems.

Key words: biological monitoring, ventilatory behavior, biomonitoring, bluegill sunfish, breathing, water quality, box plots, line plots

SYDOR, W. J., W. R. MILLER III, J. CAIRNS JR., AND D. GRUBER. 1982. Use of box and line plots to assess fish ventilatory behavior in biological monitoring systems. Can. J. Fish. Aquat. Sci. 39: 1719 – 1722.

Des signaux de ventilation émis par 12 crapets arlequins (*Lepomis macrochirus*) ont été captés à l'aide d'un mini-ordinateur en direct dans le cadre d'un programme de surveillance biologique. Les résultats de trois expériences démontrent que ces techniques graphiques peuvent être appliquées à des systèmes de surveillance biologique en direct.

Received September 2, 1981
Accepted August 13, 1982

Reçu le 2 septembre 1981
Accepté le 13 août 1982

THE purpose of this study was to examine the use of box and line plots for assessing fish ventilatory behavior in biological monitoring systems. Biological monitoring is a means of assessing environmental quality using living organisms as sensors of environmental perturbations (Cairns and van der Schalie 1980). Biological monitoring systems may be coupled to computer systems to provide for automated and continuous assessment of water quality (Cairns and Gruber 1979). The use of computer systems simplifies data analysis and markedly reduces time required for information generation. Such biological monitoring systems thus have direct industrial applications for protecting the aquatic environment from hazardous discharges.

A major thrust in the development of these biological monitoring systems has been the use of fish ventilatory rates as indicators of water quality. A variety of analytical procedures has been applied in an attempt to relate increases or decreases of fish ventilatory rates to the presence of acute or subacute concentrations of toxic substances (Cairns and Gruber 1980). Biological data are frequently highly variable and, as a consequence, subtle responses to stress may not be detected. Box plots (Tukey 1977; McGill et al. 1978) provide a technique for condensing data within a sample such that both the central tendency and its variability can be depicted graphically. A series of observations can be broken into subsamples containing the same number of equal-sized time intervals, box plots drawn for each subsample, and the resulting sequence of box plots examined to note changes over time. When long sequences are examined, each box plot can be condensed to a single line that is known as a line plot.

Methods — Ventilatory signals from 12 bluegill (*Lepomis macrochirus*) sunfish, individually housed within 5-L monitoring chambers, were received through a noncontact, submerged electrode. These signals were amplified, filtered, and interfaced to an on-line minicomputer system employing techniques described in detail elsewhere (Gruber et al. 1977, 1980). The monitoring chambers were each isolated within a compartmentalized module and provided with a diluent and/or simulated effluent via a dilutor apparatus (Gruber et al. 1979). Four fish served as controls and were exposed to the diluent exclusively. The remaining eight fish were exposed to a predetermined and metered dilution of a simulated effluent.

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Printed in Canada (J6619)
Imprimé au Canada (J6619)

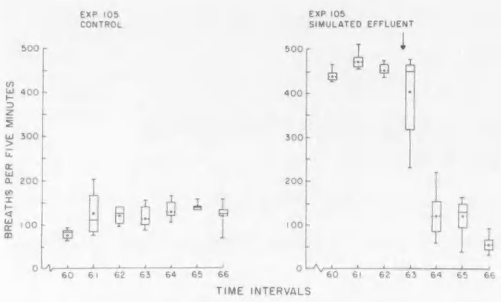

FIG. 1. Box plots of ventilatory rates vs. time for two bluegill (*L. macrochirus*) sunfish, one under control conditions and one exposed to a simulated munitions effluent. Each box summarizes data for 10 time intervals, enumerated from initiation of the experiment, of 5-min duration; i.e. time interval 60 on the horizontal axis indicates data collected between minutes 3001 and 3050 of the experiment. The top, internal, and bottom line of each box correspond to the 75, 50 (median), and 25% points, respectively, of the observed data distribution. The plus sign indicates the location of the sample mean. The vertical lines extending upward and downward from the boxes indicate the largest and smallest data values.

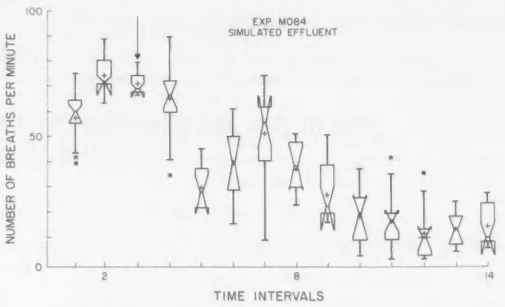

FIG. 2. Box plots of ventilatory rates vs. time for a typical bluegill (*L. macrochirus*) sunfish, before and during exposure to a simulated effluent from an ammunition manufacturing plant. Arrow indicates time during which effluent was first introduced. Each box summarizes data for 10 time intervals, enumerated from initiation of the experiment, of 1-min duration; i.e. time interval 2 summarizes data between minutes 11 and 20. The top, internal, and bottom horizontal lines of each box correspond to the 75, 50 (median), and 25% points, respectively, of the observed data distribution. The plus sign indicates the location of the sample mean. The notches in the sides of the boxes indicate approximate confidence intervals (see text for derivation). The vertical line extending upward from each box indicates the smaller of the largest observation or $1.5(R)$ above the median, where R is the difference of the 75 and 25% points. The vertical line extending down from the box indicates the larger of the smallest observation or $1.5(R)$ below the median. The asterisks "*" indicate outliers, observed data that are larger or smaller than the median $\pm$ $1.5(R)$.

Ventilatory rates were collected in 1- or 5-min time intervals and stored by the minicomputer system. Although the computer system has the capability of detecting significant variations in ventilatory rates with a time series based algorithm, the original data sets were utilized for detecting changes in ventilatory rates via box and line plots. Initially, data were collected for other purposes and consisted of the number of breaths per each 5-min time period; however, applications of the box plot technique to such data only allowed identification of the most gross changes in ventilatory behavior. Some experiments were completed using time periods of 1-min duration to determine if this would improve the sensitivity of the analysis.

Three different experiments were performed. In the first and second experiments, eight fish were exposed to a simulated ammunition plant's untreated wastewater. Although this simulated wastewater included a variety of constituents, the concentration of nitroglycerin [reported to have an acute LC50 for *L. macrochirus* of 1.7 $mg \cdot L^{-1}$ (Sullivan et al. 1978)] was of particular concern. In the third experiment, eight fish were exposed to a sodium hypochlorite solution increasing in concentration over time to 0.50 $mg \cdot L^{-1}$ total residual chlorine (TRC).

A typical sequence of box plots is presented in Fig. 1 and provides information regarding several important characteristics of the data. The bottom, middle, and top lines of each box correspond to the 25, 50 (median), and 75% points of the data distributions based on the computational procedure of McGill et al. (1978). The 25% point is the observed data value which is larger than or equal to 25% of the observed values. It is also the value which is smaller than or equal to 75% of the observations. The 50 and 75% points are defined in an analogous fashion. The plus sign (+) in the interior of each box corresponds to the mean of the sample data. The mean and median ventilatory rates are not necessarily equal, and the sample mean can fall outside the limits of the 25 and 75% points. The horizontal bars at the ends of the lines (sometimes called "whiskers") extending up and down from the box indicate the largest and smallest observed values, respectively.

In an examination of data, it may be desirable to determine whether two box plots are significantly different from one another or whether the differences can be attributed to natural variation. For independent observations, McGill et al. (1978) suggest that an approximate 95% confidence interval can be obtained for the median from the following expression:

$$M \pm 1.7(1.25\,R/1.35\sqrt{N})$$

where M is the observed median value, R is the difference between the 75 and 25% points, and N is the size of the sample used in forming the box plot. Notches are then formed in the sides of the boxes (Fig. 2) to indicate this approximate confidence interval. These notches may extend beyond the ends of the box. If observations are independent and the notches do not overlap, then the two sets of data may be considered significantly different at an α level of ~ 0.05.

The use of an approximate confidence interval is not strictly applicable to these data as individual data values are not independent (time series analysis has indicated significant autocorrelation among time intervals). Yet in an exploratory sense, the approach is useful. When notches do not overlap, there is an indication of possible differences that may warrant further investigation.

Outliers, observations that depart from the bulk of the other data, can also be indicated on the plots. Tukey (1977) defines

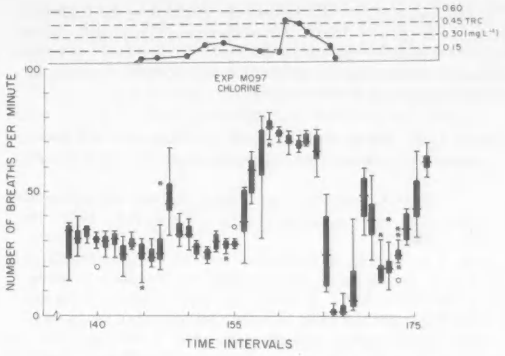

FIG. 3. Line plots of ventilatory rates vs. time for a typical bluegill (*L. macrochirus*) before and during exposure to a sodium hypochlorite solution. Total residual chlorine (TRC) in $mg \cdot L^{-1}$ has been indicated at the top of the figure. Each plot summarizes data for 10 time intervals, enumerated from initiation of the experiment, of 1-min duration; i.e. time interval 140 summarizes data between minutes 1401 and 1410. The darkened portion of each plot corresponds to the region covered by the 25 to 75% points of the data distribution. The horizontal line within this region indicates the sample median. The vertical line extending above the darkened region indicates the smaller of the largest observation or $1.5(R)$ above the median, where R is the difference between the 75 and 25% points of the data distribution. The vertical line extending downward from the darkened region indicates the larger of the smallest observation or $1.5(R)$ below the median. The asterisks "*" indicate outliers lying between $1.5(R)$ and $3(R)$ from the median, and open circles "○" indicate outliers lying further than $3(R)$ from the median.

two magnitudes of departure from most observations. If a value is greater than $1.5(R)$ but less than or equal to $3(R)$ units away from the median, the observation is termed an "outside" value (indicated by "*" in Fig. 2 and 3). If it is greater than $3(R)$ units from the median, Tukey calls this a "far out" value (indicated by "○" in Fig. 3; no "far out" values were detected in experiment 2). When outliers are present and it is desired to indicate them, the maximum length a "whisker" may attain is set at $\pm 1.5(R)$ units from the median.

When a long series of intervals is to be examined, the information contained in each box plot may be condensed into a single line. Figure 3 presents data in this fashion. Although these plots may not present as striking an image, do not indicate the location of the mean value, and do not show the approximate 95% confidence interval, their use provides an alternative form of display that allows more ventilatory rate data to be presented in the same space.

Fish ventilatory rates are highly variable. Input of a chemical may increase the ventilatory rate of one fish while decreasing the rate of another. Alternatively, the average rate may remain unchanged, but the variability about this value may change markedly. Box and line plots thus have potential for investigating changes in fish ventilatory rates as they illustrate both the central tendency and variability of observed data.

Both box and line plots have an advantage over the use of the mean and 95% confidence interval (or ± 1 SD) as both box and line plots characterize the observed data without making an assumption about its distributional characteristics. These plots are also more informative than plots of the median and range as inclusion of the 25 and 75% points better characterizes the concentration of the data about the median, and potential outliers can be identified.

Results — Box or line plots for typical fish in the three experiments are presented in Fig. 1, 2, and 3. It is recognized that presentation of data for a typical fish is not the best experimental practice; however, here the objective is to demonstrate that it is possible to identify at least some changes in fish ventilatory behavior from box and line plots. Thus for the sake of economy, only the figures needed to display the general pattern of ventilatory rate changes are presented; deviations from this pattern for other fish are noted in the text.

The first experiment lasted 101 h, i.e. 1210 five-minute time intervals. The ventilatory pattern for one control and one experimental fish is shown in Fig. 1 for time intervals 60 through 66. A simulated effluent was introduced at time interval 62.7. Water samples collected during this interval indicated a nitroglycerin (NG) concentration of $1.38\ mg \cdot L^{-1}$, ~ 80% of the 96-h LC50 for bluegill as reported by Sullivan et al. (1978). The remaining three fish in this group responded to the simulated effluent in a similar manner to that presented in Fig. 1. The second group of four fish exposed to the simulated effluent did not respond, and all had a relatively constant ventilatory rate during intervals 60 to 66. An examination of the original 5-min data set and log books reveals that the second group may not have received effluent as a result of clogging of the effluent delivery system. A possible explanation for this clogging was the heavy load of settleable solids that were frequently encountered when an effluent pump at a distance of 450 m was turned on. At times, the solids load was heavy enough to clog the floating-ball type flowmeters, effectively blocking effluent delivery to a group of fish.

The second experiment lasted for 23 h, i.e. 138 ten-minute intervals. Figure 2 depicts a series of sequential box plots for a fish exposed to a dilution of the simulated effluent. The time interval during which the NG concentration was first introduced is indicated by the arrow in this figure. During the experiment, measured NG concentrations increased from 1.4 to $3.6\ mg \cdot L^{-1}$. During time intervals 6 and 7, the ventilatory rate increased. As chemical samples of the water were not analyzed at this time, it is not known whether this represented a physiological compensation by the fish or whether this increase is a result of a decrease in the toxicant concentration. The potential recovery of fish ventilatory rates following the removal of toxic substances is currently under investigation, but results of experiments carried out by Cairns and Thompson (1980) indicate that recovery may follow such a reduction in concentration. The ventilatory rates of all exposed fish subsequently declined with increasing concentrations of the chemical. By time interval 42, six of eight fish were dead; at this time, the NG concentration was approximately twice the 96-h LC50, i.e. $3.6\ mg \cdot L^{-1}$.

The introduction of a toxic substance often results in a lowered ventilatory rate and/or increased variability about the average rate. Figure 3 illustrates a change in variability of

ventilatory rates following introduction of chlorine at time interval 149. The initial response was a decrease in ventilatory rate at a TRC concentration of 0.06 $mg \cdot L^{-1}$. Ventilatory rates and data variability increased as TRC concentrations approached 0.22 $mg \cdot L^{-1}$. Following a peak TRC concentration of 0.50 $mg \cdot L^{-1}$, ventilatory rates became quite irregular. A similar pattern was noted for the remaining seven TRC exposed fish.

Discussion and Conclusions — Box and line plots have potential for examining data from biological monitoring systems. Such plots provide a means of presenting data sets graphically so that differences between the data sets or patterns over time may be examined. The central tendency, variability, and approximate confidence intervals of these data sets may then be compared.

The results of three experiments have been presented to demonstrate the possible utility of box and line plots for identifying changes in fish ventilatory rates. Furthermore, the observed variability among fish or among days for the same fish does not complicate the analysis, one only need observe the plotted data and identify time intervals where potential changes may have occurred. Plots of several fish can then be compared to determine whether the indicated changes in ventilatory behavior are the result of individual variability or common to several exposed fish. These changes could then be compared to control organisms to assess whether the indicated change might be attributable to a factor other than the presence of the effluent.

Industrial applications of on-line biological monitoring systems for demonstrating wastewater discharge compliance already exist (Gruber et al. 1981). However, before widespread application of such systems become a reality, the testing of computer algorithms for assessing water quality through biological sensors is a necessity. It is anticipated that box and line plots can be used to summarize graphically the data collected in such systems. The feasibility of incorporating box and line plot outputs in the existing monitoring system is currently under investigation.

Drawing and analyzing the box plots by hand is time consuming and tedious. We have developed a Fortran program for graphing sequential box and/or line plots. A copy of the program listing is available from Biological Monitoring, Inc., P.O. Box 184, Blacksburg, VA 24060, USA.

Acknowledgments — We express our gratitude to the Chemical Systems Laboratory, Aberdeen Proving Ground, Maryland, for its financial support of the project from which this present endeavor originated. We are indebted to Darla Donald, Editorial Assistant, for the final processing of this manuscript.

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Ichthyophthiriasis: Immersion Immunization of Rainbow Trout (*Salmo gairdneri*) Using *Tetrahymena thermophila* as a Protective Immunogen

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WOLF, K., AND M. E. MARKIW. 1982. Ichthyophthiriasis: immersion immunization of rainbow trout (*Salmo gairdneri*) using *Tetrahymena thermophila* as a protective immunogen. Can. J. Fish. Aquat. Sci. 39: 1722 – 1725.

Four-hour bath exposure of rainbow trout (*Salmo gairdneri*) fry to *Tetrahymena thermo-*

Printed in Canada (J6789)
Imprimé au Canada (J6789)

phila ($1-2 \times 10^5$ cells/mL), or to the sheared cilia from an equal number of cells, followed by a holding time of 8–10 wk, provided protection against a challenge *Ichthyophthirius multifiliis* infection that killed 65% of nonimmunized controls. A holding time of 6 wk or less provided proportionally less protection. When, at the conclusion of the work, a chance infestation with *Ichtyobodo necatrix* occurred, surviving immunized fish were more resistant than nonimmunized fish. In contrast to the protection obtained against *I. multifiliis* with either whole cells or cilia, survivors of *I. necatrix* were more numerous among fish that were exposed to whole cell antigen than among those exposed to sheared cilia.

Key words: Ichthyophthiriasis, *Ichthyophthirius multifiliis*, *Tetrahymena thermophila*, immunization, *Ichtyobodo necatrix*, rainbow trout, *Salmo gairdneri*; immunity, immune response, ciliates

WOLF, K., AND M. E. MARKIW. 1982. Ichthyophthiriasis: immersion immunization of rainbow trout (*Salmo gairdneri*) using *Tetrahymena thermophila* as a protective immunogen. Can. J. Fish. Aquat. Sci. 39: 1722–1725.

Une exposition par immersion, pendant 4 h, d'alevins de truite arc-en-ciel (*Salmo gairdneri*) à *Tetrahymena thermophila* ($1-2 \times 10^5$ cellules/mL) ou aux cils enlevés d'un nombre égal de cellules, suivie d'une stabulation de 8 à 10 sem, protège les poissons contre une infection par *Ichthyophthirius multifiliis* qui tuait 65 % des témoins non immunisés. Une stabulation de 6 sem ou moins donne une protection proportionnellement inférieure. À la fin de l'expérience, après infestation accidentelle par *Ichthyobodo necatrix*, les poissons immunisés qui avaient survécu ont été plus résistants que les poissons non immunisés. Contrairement à la protection que donnent soit des cellules entières, soit les cils seulement contre *I. multifiliis*, les survivants de l'infection par *I. necatrix* ont été plus nombreux parmi les poissons qui avaient été exposés à l'antigène de cellules entières que parmi ceux exposés aux cils seulement.

Received January 20, 1982
Accepted July 27, 1982

Reçu le 20 janvier 1982
Accepté le 27 juillet 1982

WORLDWIDE in freshwater fish husbandry, the protozoan parasite *Ichthyophthirius multifiliis* is recognized as a significant problem in that it commonly causes mortality, and is unusually difficult to control. That difficulty arises from several features in the parasite's life cycle. Although it is short-lived and vulnerable to an array of practical control measures, the invasive or infective stage (tomite) is diverse in the time of its appearance, and within a population of fish can be produced and available to infect on a continual basis. In contrast and time-wise, most of the parasite's life cycle is spent within host epithelium where it is apparently unaffected by chemotherapeutants added to fish feeds and is certainly not exposed to chemical solutions applied externally by methods used in fish husbandry.

Immunization holds promise as a practical and effective control measure for ichthyophthiriasis. Buschkiel (1910) might have been the first to raise the question of fish immunity to *I. multifiliis*. He noted that when fish survived the infection, they showed reduced susceptibility to later challenge with the parasite. Later workers made similar observations. Hines and Spira (1974) used quantified sublethal infestations of *I. multifiliis* to evoke an immune response in common carp (*Cyprinus carpio*) that protected them for at least 8 mo against challenges that killed all nonimmunized fish.

Methods for culturing *I. multifiliis* in vitro have not been developed. Accordingly, stocks of the parasite must be maintained by passage in living susceptible hosts (Hines and Spira 1974; Goven et al. 1981). Because of problems inherent in maintaining an obligate parasite for use as an immunogen, alternative sources of a related but heterologous antigen were explored (Goven et al. 1980, 1981).

Goven et al. (1980, 1981) investigated *Tetrahymena pyriformis*, a cultivable, free-living, but facultatively parasitic ciliate, and showed that intraperitoneal injection of cilia from *T. pyriformis* protected channel catfish (*Ictalurus punctatus*) against challenge with lethal ichthyophthiriasis. However, deciliated cells of *Ichthyophthirius multifiliis* or *T. pyriformis* were not protectively antigenic. In vitro serologic tests confirmed that the two ciliates were antigenically related and that antigenicity was largely in the cilia.

We undertook to determine whether *T. thermophila*, an easily cultured, free-living but nonparasitic ciliate, could serve as a protective immunogen against experimental ichthyophthiriasis in rainbow trout (*Salmo gairdneri*). We sought to determine the optimum response time for the induction of significant levels of protection and to compare the antigenic potency of whole cells with that of cilia alone.

Our exploratory work (unpublished) showed that exposure by immersion to *T. thermophila* antigen needed to be for 1 h or longer; the induction period ought to extend for at least several weeks; and *T. thermophila* did indeed offer promise as an immunogen against *I. multifiliis*.

Materials and Methods — Fish — We used fry of Fall Standard No. 15 strain rainbow trout of the U.S. Fish and Wildlife Service fish genetics program. The fry were 2 mo old at the time the first group was exposed to *T. thermophila*. Before and during the time for induction of an immune

response, the fish were kept at 12.5°C. The chemical properties of our water supply were reported by Warren (1963).

Tetrahymena thermophila — Strain WH6, ATCC No. 30 007 was obtained from the American Type Culture Collection and grown in a 1:10 dilution of their medium No. 383 but without the agar (8 g dextrin, 0.6 g sodium acetate, 5 g yeast extract, 0.6 g liver extract, 5 g tryptone, 1 L water). The pH was adjusted to 6.2−6.4. Incubation at room temperature for 5−6 d yielded a population density of $1.5-2.0 \times 10^5$ cells/mL. When needed for whole cell antigen, living cells were sedimented at 500 × *g* for 3 min at 4°C, and then resuspended in water to give a cell density of $1-2 \times 10^5$/mL. The organisms were deciliated either with dibucaine according to the procedure of Thompson et al. (1974), or with ethylenediaminetetraacetate by the method of Rosenbaum and Carlson (1969). Sheared cilia were stored in water at −20°C until used.

Immunization — Twenty fry were exposed for 4 h at 12.5°C, with aeration to 500 mL of either live whole cells ($1-2 \times 10^5$ cells/mL) or to the cilia sheared from an equal number of cells. Two weeks later, the fish received a second exposure to the same procedures. Samples of 20 fry each were exposed at 2-wk intervals to give a range of immunization time of 2−10 wk before challenge. Control fish were not exposed to *T. thermophila* cells or cilia.

Challenge with I. multifiliis — Stocks of *I. multifiliis* were obtained from goldfish (*Carassius auratus*) and rainbow trout, and maintained at 12 and 15°C in rainbow trout fingerlings.

For uniformity of challenge with *I. multifiliis*, each group of 20 test fish was held with eight lightly infected and finclipped fish in a compartment of a partitioned trough. Water was heated to 15°C, aerated, and supplied at a rate of six changes per hour. Surviving infected source fish were removed at the end of a week.

Results — Immunization with *T. thermophila* provided protection against *I. multifiliis* (Table 1). After a week's exposure to *I. multifiliis*, 4 wk elapsed before the first mortality occurred among the test fish. The disease ran its course in 3 wk and resulted in the death of two-thirds of the nonimmunized controls (Table 1). Among the immunized fish there was a nearly linear relation between survivors of the disease and the duration of immune induction time. Results were best when the period of immune induction was 8−10 wk (Table 1), but there was no significant difference between the efficacy of whole cell antigen and sheared cilia antigen ($\chi^2 = 9.54$, $P = \sim 0.5$).

The correlation coefficient of the data for induction time and survival was 0.89, a highly significant value.

An unexpected outbreak of *Ichtyobodo necatrix* occurred at termination of the *Ichthyophthirius multifiliis* work (start of the 8th wk postchallenge). Losses were significantly greater among fish that had been immunized with sheared cilia than among those immunized with whole cells ($\chi^2 = 32.49$, $P = <0.005$). In the groups immunized with whole cell antigen, deaths due to *I. necatrix* were greatest among groups that had been immunized for the shortest periods (Table 1).

TABLE 1. Comparative mortality of rainbow trout fingerlings due to *Ichthyophthirius multifiliis* or *Ichtyobodo necatrix* following immunization by immersion in suspension of whole live cells or cilia of *Tetrahymena thermophila*.

Time from initial exposure (wk)	Mortality from *I. multifiliis* (no. dead/%)	Mortality due to *I. necatrix* (%)
	Whole cell antigen	
0	13/65[a]	86
2	9/45	55
4	2/10	72
6	3/15	47
8	1/5	5
10	0/0	10
	Ciliary antigen	
0	14/70	83
2	5/25	87
4	3/15	94
6	2/10	61
8	2/10	53
10	3/15	65

[a]The starting number for each group was 20.

The survival of fish that had been immunized with whole cell antigen and survived *I. necatrix* also was significantly higher than that of unimmunized controls ($\chi^2 = 32.49$, $P = <0.001$). As in ichthyophthiriasis, survival after exposure to *I. necatrix* was highest in groups originally immunized with whole cell antigen for 8 or 10 wk.

Discussion — Our results provide evidence that bath immersion of young trout in either a suspension of whole cells or of sheared cilia of *T. thermophila* provided protection against ichthyophthiriasis. Evidence that immunization against *Ichthyophthirius multifiliis* also provided protection against *Ichtyobodo necatrix* was unexpected and suggests that cross-protection against other parasitic ciliates, e.g. *Chilodonella* and *Trichodina*, might also result. Hines and Spira (1974) found that common carp immunized against ichthyophthiriasis could be parasitized by *Trichodina*, but the degree of involvement was described as only "moderate."

Although the respective fish species, immunogens, and methods of vaccination differed, our results are similar to those obtained by Goven et al. (1980).

Practical application of immunization with *T. thermophila* appears promising, but larger experimental trials must be performed. The effect of temperature on the immune response needs to be determined, as well as longevity of protection. In that regard, it seems likely that when immunized fish encounter *I. multifiliis*, a secondary immune response could be evoked. If so, the effect could enhance protection and be of considerable advantage in husbandry.

Practical applications of immunization by immersion will require large quantities of antigen. *Tetrahymena thermophila* is easily grown and can be efficiently used if fish are immunized as fry.

Acknowledgment — We thank R. C. Simon, National Fish Health Research Laboratory, Kearneysville, for conducting the statistical analyses of our data.

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Characteristics and Identification of *Pasteurella* and *Vibrio* Species Pathogenic to Fishes using API-20E (Analytab Products) Multitube Test Strips

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KENT, M. L. 1982. Characteristics and identification of *Pasteurella* and *Vibrio* species pathogenic to fishes using API-20E (Analytab Products) multitube test strips. Can. J. Fish. Aquat. Sci. 39: 1725–1729.

Morphological, cultural, and biochemical characteristics of *Pasteurella* spp. and *Vibrio* spp. pathogenic to fishes are investigated. *Vibrio anguillarum*, *V. ordalii*, *V. alginolyticus*, *V. parahaemolyticus*, an unidentified *Vibrio* sp. (AN1K), *Pasteurella piscicida*, and *P. multocida* were cultured on API-20E multitube test strips (Analytab Products) and in Baumann's marine media with similar results. The API-20E Profile Index was not useful for classification of fish bacteria studied and, using the index, *Vibrio* sp. AN1K was misidentified as *P. multocida*. Bacteria studied were also grown on Marine Agar (Difco) and on blood agar at 25 and 37°C. On blood agar at 37°C some vibrios resembled pasteurellas as a result of pleomorphism and lack of motility. Simple diagnostic procedures for identifying *Pasteurella* spp. and *Vibrio* spp. associated with fishes using the API-20E with modifications are presented.

Key words: *Pasteurella*, *Vibrio*, characteristics, Analytical Profile Index, fish pathology

KENT, M. L. 1982. Characteristics and identification of *Pasteurella* and *Vibrio* species pathogenic to fishes using API-20E (Analytab Products) multitube test strips. Can. J. Fish. Aquat. Sci. 39: 1725–1729.

Les caractéristiques morphologiques, culturales et biochimiques de *Pasteurella* spp. et *Vibrio* spp., organismes pathogènes des poissons, ont été étudiées. On a cultivé *Vibrio anguillarum*, *V. ordalii*, *V. alginolyticus*, *V. parahaemolyticus*, une espèce de *Vibrio* non identifiée (AN1K), *Pasteurella piscicida* et *P. multocida* sur bandes multitubes API-20E (Analytab Products) et dans le milieu marin de Baumann, avec résultats identiques. L'indice de profil avec API-20E s'est avéré inutile pour classifier les bactéries de poissons étudiées: avec cet indice, *Vibrio* sp. AN1K a été incorrectement identifié comme *P. multocida*. Les bactéries étudiées ont également été cultivées sur gélose marine (Difco) et gélose sanguine à 25 et 37°C. Sur ce dernier milieu à 37°C, certains vibrions ressemblent aux pasteurellas à cause de leur pléomorphisme et l'absence de motilité. Nous décrivons des méthodes diagnostiques simples pour l'identification de *Pasteurella* spp. et *Vibrio* spp. associés aux poissons, en utilisant API-20E avec modifications.

Received May 3, 1982
Accepted August 30, 1982

Reçu le 3 mai 1982
Accepté le 30 août 1982

Printed in Canada (J6920)
Imprimé au Canada (J6920)

VIBRIOSIS is considered by many to be the most important disease of saltwater-reared salmonids (Sindermann 1974) and is cosmopolitan, with outbreaks occurring in many fish families in the Americas, Asia, and Europe (Anderson and Conroy 1970). The etiological agents of fish vibriosis are *Vibrio anguillarum* and *V. ordalii* (Scheiwe et al. 1981), although *V. parahaemolyticus* has also been incriminated (Bullock 1971). *Vibrio anguillarum, V. parahaemolyticus*, as well as *V. alginolyticus* are reported pathogens of cultured invertebrates (Sindermann 1974). Pasteurellosis, caused by *Pasteurella piscicida,* has been reported in striped bass (*Morone saxatilis*) and white perch (*M. americana*) (Sniesko et al. 1964), and *P. plecoglosacida* has caused disease in cultured ayu (*Plecoglossus altivelis*) in Japan (Kusuda and Miura 1972). Some isolates from freshwater fishes were identified as *Pasteurella* spp. (Ajmal et al. 1967; Hastein and Bullock 1976) but were later found to be atypical *Aeromonas salmonicida* strains by Paterson et al. (1980), who also confirmed the results of Jassen and Surgalla (1968) that the *P. piscicida* from *Morone* spp. (Sniesko et al. 1964) was not an *Aeromonas* sp.

Both *Vibrio* and *Pasteurella* cause septicemic infections and in acute cases few pathological changes may occur. Diagnosis must therefore be based on the isolation and identification of the suspected pathogen. As both genera share many morphological and biochemical characteristics, they may be difficult to distinguish. Both are Gram-negative rods, often exhibit pleomorphism, are oxidase-positive, and ferment sugars without the production of gas. Most vibrios can be distinguished from *Pasteurella* by their motility, production of gelatinase, and lack of bipolar staining, but sometimes these characteristcis are variable. Confusion is added to the differentiation of the two genera due to an apparently new *Vibrio* sp. isolated from dying jacks at the Scripps Aquarium. This motile bacterium does not produce gelatinase and has other biochemical characteristics identical with *P. multocida*.

The purpose of the present study was to compare morphological, cultural, and biochemical characteristics of a possibly new *Vibrio* sp. (AN1K) with *Vibrio* spp. and *Pasteurella* spp. pathogenic to fishes. Biochemical characteristics of *P. piscicida* and *Vibrio* spp. associated with fishes are obtained using API-20E multitube test strips and are compared to those using media designed for identification of marine bacteria by Baumann and Baumann (1981). Using these results, simple diagnostic methods for identifying these bacteria using the API-20E are presented.

Materials and methods — Bacterial strains — Morphological, biochemical, and cultural characteristics of the following strains were investigated: *P. piscicida* strains ATCC 17911 and ATCC 29687; *P. multocida* (four strains isolated from rabbits); *V. anguillarum* strains ATCC 16294 and 273 (obtained from Dr J. F. Steenbergen, San Diego State University); *V. anguillarum* strains V-1, LS1-74, and 775, and *V. ordalii* strains DF3K, C74-48, and YAV-503 (from Dr M. H. Schiewe, Northwest and Alaska Fisheries Center, Seattle, WA); *V. alginolyticus* strains 118, 155, 90, and 86, and *V. parahaemolyticus* strain 133 (from Dr K. Nealson, Scripps Institution of Oceanography, La Jolla, CA); and *Vibrio* sp. AN1K isolated at our laboratory from the livers and kidneys of dying jacks (*Chloroscombrus* sp.) held at the Scripps Aquarium, Scripps Institution of Oceanography. Strains of *P. plecoglosacida* were not available and reported characteristics were obtained from Kusuda and Miura (1972).

Morphology, motility, and cultural conditions — Gram character and bipolar staining were determined using standard methods described by Benson (1979). Morphology and motility were determined with wet mounts in 0.9% saline or 50% artificial seawater using cultures incubated at 25 and 37°C on Marine Agar (Difco) and 6% Sheep Blood Agar (Clinical Standards Laboratories). The ability to grow at 40°C was tested on marine agar in a National incubator. Flagellation was determined using the Leifson flagellar stain with modifications described by Baumann and Baumann (1981).

Biochemical characteristics — Sensitivity to 0/129 Vibriostat (2:4 diamino 6:7 DI isopropyl pteridine) was performed using disks impregnated with 10μg as described by Bullock (1971). Sensitivity to penicillin was determined using disks impregnated wth 30 μg (Difco). API-20E multitube test strips (Analytab Products, Plainview, NY) were used to determine biochemical characteristics shown in Table 1. The following tests were also performed on the marine species using media designed for marine bacteria by Baumann and Baumann (1981): acid from glucose, mannitol, sorbitol, and sucrose; NO_3 to NO_2; citrate utilization; arginine dihydrolase, and the production of gelatinase. All procedures described by Analytab Products for using the API-20E were followed except the marine species were inoculated onto the strips using 50% artificial seawater made according to Baumann and Baumann (1981), and all cultures were incubated at 25°C for 48 h. The requirement of sodium for growth was determined using MR−VP broth (Clinical Standards Laboratories). Only bacteria not requiring sodium will grow in this sodium-deficient medium (Blake et al. 1980). The production of oxidase was determined using standard methods with a 1.0% solution of dimethyl-*p*-phenylenediamine hydrochloride (Benson 1979).

Results — Morphology, motility, and cultural characteristics — Both *Pasteurella* spp. and *Vibrio* spp. exhibited pleomorphism. All *Pasteurella* spp. were nonmotile and formed Gram-negative pleomorphic rods and coccibacilli. *Pasteurella piscicida* grew slowly on marine agar and blood agar at 25°C and did not grow at 37°C. *Pasteurella multocida* grew under all conditions and growth was slowest on marine agar at 25°C. All *V. anguillarum* strains grew and exhibited pleomorphism under all conditions. Pleomorphism was more prominent in older cultures, particularly when grown at 37°C on blood agar. *Vibrio anguillarum* strains V-1 and LS1-74 grew slowly on blood agar at 37°C and motility was not observed after 36 h. All other *V. anguillarum* strains maintained motility at least 60 h on blood agar at 37°C, but less than 1% of the cells were motile. *Vibrio ordalii* grew only at 25°C on marine agar and blood agar. Motile, straight, and pleomorphic rods were observed under these conditions for 60 h. *Vibrio alginolyticus* and *V. parahaemolyticus* grew rapidly and formed straight and pleomorphic motile rods in all conditions. As with *V. anguillarum*, pleomorphism was more prominent in cultures grown at 37°C on blood agar. *Vibrio*

TABLE 1. Biochemical characteristics of *Vibrio* and *Pasteurella* spp. grown at 25°C for 48 h on API-20E test strips and Baumann's media.

Species	*P. piscicida*	*P. plecoglossida*[a]	*P. multocida*	*Vibrio* AN1K	*V. anguillarum*	*V. ordalii*	*V. alginolyticus*	*V. parahaemolyticus*
Sodium requirement	+[b]	+	−	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+
Sensitivity:								
0/129	+	n.r.[c]	+	+	+	+	−	−
Penicillin	+	n.r.	+	−	−	−		−
Novobiocin	+	n.r.	−	+	+	+	+	+
NO_3-NO_2	−	−	+	+	+	+	+	+
ONPG	−	n.r.	−	−	+	−	−	−
Arginine dihydrolase	+	−	−	−	V[d]	−	−	−
Lysine dicarboxylase	−	−	−	−	−	−	+	+
Ornithine dicarboxylase	−	−	−	−	−	−	+	+
Citrate utilization	−	n.r.	−	−	+	−	+	+
Urease	−	n.r.	−	−	−	−	−	−
Trytophane deaminase	−	n.r.	−	−	−	−	−	−
Indol	−	−	+	+	V[e]	−	+	+
Voges−Proskauer	−	−	−	−	+	−	+	−
Gelatinase	−	−	−	−	+	+	+	+
Acid from:								
Glucose	Weak+	+	+	+	+	+	+	+
Mannitol	−	−	+	+	+	+	+	+
Inositol	−	n.r.	−	−	−	−	−	−
Sorbitol	−	n.r.	+	+	+	−	−	−
Rhamnose	−	n.r.	−	−	−	−	−	−
Sucrose	−	+	+	+	+	+	+	−
Melibiose	−	−	−	−	−	−	−	−
Amygdalin	−	n.r.	−	−	+	−	−	−
Arabinose	−	−	−	−	V[f]	−	−	+
Growth at 40°C	−	−	+	−	−	−	+	+

[a]Data for *P. plecoglossida* from Kusuda and Miura (1972).
[b]+, all strains positive; −, all strains negative; V, variable.
[c]n.r., not reported.
[d]Strains V-1 and LS1-74 were ADH-negative on the API-20E and ADH-positive in Baumann's media.
[e]Strain 775 was indol-negative.
[f]Strains V-1 and ATCC 16294 were arabinose-negative, all other strains were positive.

sp. AN1K grew at 25 and 37°C on both media and motility was observed in all conditions except when grown on blood agar at 37°C. Under this condition the *Vibrio* sp. AN1K was nonmotile and formed pleomorphic rods after 24 h. The ability of this bacterium to grow at 37°C on blood agar was lost after six culture transfers.

Flagella were observed only on the *Vibrio* spp. and all were polarly flagellated. Many unattached flagella were seen and observations of large areas of the slides were required to reveal bacteria with flagella attached. Bipolar staining was observed only in *P. piscicida*. *Pasteurella multocida* did not exhibit bipolar staining and cultures had been transferred three times before staining. No *Vibrio* spp. exhibited bipolar staining.

Biochemical characteristics — Biochemical characteristics are shown in Table 1. Arginine dehydrolase activity was observed in both strains of *P. piscicida*, but strain ATCC 29687 required 72 h before a positive reaction was evident with either the API-20E test or in Baumann's ADH medium. *Vibrio anguillarum* strains V-1 and LS1-74 were arginine dehydrolysis-negative on the API-20E and were positive in Baumann's ADH medium. All other *V. anguillarum* strains were positive in both media. All other biochemical reactions of the bacteria studied on the API-20E and in Baumann's media were the same. Positive carbohydrate fermentations by the vibrios on the API-20E produced a yellow color while, as reported by Collins and Swanson (1981), the *Pasteurella* spp. produced a yellow-green color which indicated weak acid production. All bacteria from marine sources exhibited a sodium requirement for growth. *Vibrio anguillarum* required the lowest concentration, and strains 273 and LS1-74 grew in MR−VP broth when transferred from marine agar. Growth did not occur when these strains were transferred from

TABLE 2. Characteristics useful for the identification of piscine *Pasteurella* spp. and *Vibrio* obtainable using the API-20E system.

	P. piscicida	*P. plecoglossida*	*P. multocida*	*Vibrio* AN1K	*V. anguillarum*	*V. ordalii*	*V. alginolyticus*	*V. parahaemolyticus*
Gelatinase	−[a]	−	−	−	+	+	+	+
Sorbitol	−	−	+	+	V[b]	−	−	−
Sucrose	V	+	+	+	+	+	+	−
Arabinose	−	n.r.	−	−	V[b]	−	−	+
VP[c]	−	−	−	−	+	−	+	−
Sensitive to penicillin	+	n.r.	V	−	−	−	−	−
Motility at 20−25°C	−	−	−	+	+	+	+	+
Growth at 40°C	−	−	+	−	−	−	+	+
Oxidase	+	+	+	+	+	+	+	+

[a] +, positive; −, negative; V, variable; n.r., not reported.

[b] Baumann and Baumann (1981) reported 90% of *V. anguillarum* strains positive for sorbitol and 80% positive for arabinose.

[c] VP reagents should be fresh. Many vibrios reported to be VP-positive appear to be negative on API-20E test strips.

MR−VP to fresh MR−VP broth.

Identifications of the bacteria studied using results from the API-20E test strips and the API-20E Profile Index are as follows: *V. anginolyticus* strains 86 and 90 were identified correctly and strains 155 and 118 were not identified; *V. parahaemolyticus* and *P. multocida* strains were identified correctly; *V. anguillarum*, *V. ordalii*, and *P. piscicida* strains were not identified; and *Vibrio* sp. AN1K had the same index number as *P. multocida* (0044524) and would be misidentified as this species if grown on the API-20E and blood agar.

Discussion — *Pasteurella* spp. and *Vibrio* spp. were found to have many characteristics in common and could be confused if isolates are grown in conditions in which *Vibrio* spp. lose motility. In agreement with Blake et al. (1980), Gillespie and Timoney (1981), and Simdu and Egusa (1972), cell morphology, bipolar staining, and sensitivity to 0/129 Vibriostat were inconsistent and therefore not useful criteria for differentiating the bacteria studied.

As reported by Stevenson and Daly (1982), API-20E test strips provided a convenient tool for examining fish isolates. However, misidentifications can occur when the API-20E Profile Index is used with the test strips. *Vibrio* sp. AN1K was misidentified as *P. multocida* and most other *Vibrio* spp. were not identified. Though the index is not useful, the API-20E test strips satisfactorily recognized important biochemical characteristics of the bacteria studied and have also been used successfully at 20−25°C by Laurencin and Tangtrongpiros (1980) to identify *V. anguillarum*, and Stevenson and Daly (1982) to identify *Yersinia ruckeri*. With few exceptions all API-20E results agreed with those obtained using Baumann's media for marine bacteria and agreed with those reported by Baumann and Baumann (1981), Lee et al. (1978), and Scheiwe et al. (1981) for *Vibrio* spp., and Koike et al. (1975) for *P. piscicida*.

The biochemical characteristics of *Vibrio* sp. AN1K were different from those reported for all other *Vibrio* spp. by Baumann and Baumann (1981) and this bacterium is being further investigated by Dr R. R. Colwell, University of Maryland.

Given the results from this study, the following tests and procedures for identifying *Vibrio* and *Pasteurella* spp. associated with fishes using the API-20E are suggested. Materials are all commercially available and results should be clear and simple to ascertain. Bacteria from marine organisms should be isolated on Marine Agar (Difco) and cultured at room temperature. Blood agar should be adequate for freshwater isolates. Baumann and Baumann (1981) described special media for identification of marine bacteria. These media are made from "scratch" and would only be appropriate for laboratories investigating large numbers of marine isolates. Instead, for practical purposes, I recommend using the API-20E test strips for identifying fish vibrios, and *Pasteurella* spp. API-20E should be inoculated with cells suspended in 50% sterile artificial seawater. Commercial dehydrated artificial seawater supplied by aquarium fish stores is satisfactory. All cultures should be grown at 20−25°C and read after 48 h. Some vibrios, such as *V. alginolyticus* and *V. parahaemolyticus*, grow rapidly, and tests can be read sooner. All other procedures described with the API-20E kit should be followed. The Profile Index provided with the kit should not be used as a result of possible misidentifications. Biochemical and morphological characteristics which can be obtained from the API-20E and marine agar plates which are useful for identifying fish-pathogenic *Vibrio* and *Pasteurella* are found in Table 2. Lee et al (1978), Baumann and Baumann (1981), Blake et al. (1980), and Schiewe et al. (1981) provide exten-

sive lists of the biochemical characteristics of the genus *Vibrio*. Bullock (1978) and Koike et al. (1975) reported the biochemical and morphological characteristics of *Pasteurella* spp. from fishes.

Acknowledgment — I thank Dr L. C. Corbeil, Dr B. B. Hemmingsen, Dr M. H. Schiewe, and Joe Lyons for helpful suggestions and for reviewing the manuscript.

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Functional Adaptations of Marine Organisms. *F. John Vernberg and Winona B. Vernberg, ed. Physiological Ecology Series. Academic Press, New York, NY, and Academic Press Canada, Don Mills, Ont. 1981. 347 p. $51.35.*

With a team of contributing authors, the editors have undertaken a truly demanding task: ". . . . to provide an insight into some of the functional adaptations of marine organisms to both natural and man-made sets of factors." In the bounds of a single volume it would be impossible to cover the field comprehensively, and the authors, accordingly, have been selective. To biologists in general the term "adaptation" denotes an adjustment to environmental conditions. It is used in at least two widely different senses. First, for the modification of a population or species through evolution which makes it more fit for the environment in which it lives (phylogenetic adaptation) and, secondly, for the physiological responses of an individual to immediate environmental stimuli (adaptive modification). With neither a consistent nor a clear distinction between these two phenomena, the authors have chosen to concentrate on the latter. For this reason, the book belies the promise of the title.

The material is divided into chapters along quasi-ecological lines, Primary Producers, Zooplankton, Macrobenthic Fauna, and so on. Within each chapter there is a brief survey of reported responses to such environmental factors as temperature, salinity, and light intensity, together with some observations on assimilation, reproduction, and life histories. This format tends to be a little repetitive. The authors seldom overtly demonstrate the way in which an observed response is, in fact, adaptive. The text is a rich compendium of examples but poor in explanation of mechanisms. A conceptually clearer approach might have been to analyze the physical and biotic aspects of the environmental phenomenon under discussion, to review the fundamental mechanism(s) of adaptation to it, which are common to all organisms and only then to explore some of the more interesting examples in the literature. For example, the biological implications of temperature fluctuations and the mechanism of temperature acclimation might be discussed in general terms. The subject could then be profitably developed by exploring the physical differences between such habitats as intertidal and subtidal sediments and by examining the ways in which closely related species such as *Tellina tenius* and *T. fabula* (p. 183) achieve their different tolerances.

The almost total neglect of fundamental adaptations in structure and form is surprising. There is a well-documented and widely recognized relationship between size, shape, and many physiological processes. This is briefly acknowledged in the discussion of diatoms where smallness and shape are treated as adaptive features. The treatment of Meiofauna would have been greatly enhanced if the peculiar problems of small size, interstitial existence, and the characteristics of sediment beds had been considered. Similarly, there is no exploration of the phenomena associated with water flow or of movement through water. The application of fluid mechanics in biology, although a relatively recent development, is an active field of research today. It is scarcely to be doubted that hydrodynamic forces have constituted one of the most important factors in the adaptation of marine organisms. It is adaptation to these forces which confers the characteristic shape of many organisms and underlies much of their behavior and physiology. Timko (O'Neill), for example, has recently shown that the Pacific sand dollar uses totally different feeding mechanisms in the horizontal and upright postures (Biol. Bull. 151: 247–259, 1975). In the latter position individuals exploit the lift properties of the curved surface in a group facilitation of feeding (Oecologia 34: 157–174, 1978). Numerous other examples could be cited that illustrate the inextricable relationship between the fundamental form of an organism and its respiration, feeding, locomotion, gamete release, and, indeed, all of its vital processes.

For the evolutionary biologist and those interested in structure and form, this book will be disappointing. For the ecologist and physiologist it provides a review of some of the literature on physiological responses to environmental stimuli. Few of the chapters, however, cite primary sources of the last 5 years. Given these limitations it is not surprising that the authors have only partially realized their stated objective.

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Books received/Livres reçus[1]

August/août 1982

Netting Materials for Fishing Gear. *Gerhard Klust. Second edition. An FAO Fishing Manual. Published by arrangement with the Food and Agriculture Organization of the United Nations by Fishing News Books Ltd., Farnham, Surrey, England. 1982. 192 p. £ 8 plus 80 p post/packing.* Many modifications and additions have been incorporated to update the original book especially revised data on the materials used in different types of nets. Practical advice on how to ensure that the correct fiber is chosen and that a specification produces the desired result is included. [R446]

The Yankee Mariner & Sea Power: America's challenge of ocean space. *Edited by Joyce J. Bastell, Published by University of Southern California Press, Los Angeles. Distributed by Transaction Books, Rutgers University, New Brunswick, NJ. 1982. 297 p. $20.* Papers from a conference sponsored by Center for Study of the American Experience, The Annenburg School of Communications, University of Southern California, March 1981. The authors' report is a broad-based guide to America's past and present sea power stance and to the requirements for her future challenge of ocean space. [R447]

Revision of the Fish Genera *Gymnelus* Reinhardt and *Gymnelopsis* Soldatov (Zoarcidae), with Two New Species and Comparative Osteology of *Gymnelus viridis*. *M. Eric Anderson. National Museum of Natural Sciences Publications in Zoology, Number 17, National Museums of Canada, Ottawa. 1982. 76 p.* [R449]

Effects of SO_2 and Its Derivatives on Health and Ecology. In four parts: Preface and executive summary; Volume 1 (seven chapters), Human health; Volume 2 (six chapters), Natural ecosystems: agriculture, forestry and fisheries; Technical summary of working group reports. *Report of a working group sponsored by the International Electric Research Exchange, Palo Alto, California. 1981. Freely available for cost of postage.* Concerns a critical evaluation of a very large volume of scientific work of import to industrialized nations. Intended for use by working scientists as well as by policy makers in industry and government. [R450]

Descriptive Physical Oceanography: an introduction. Fourth enlarged edition (in SI units). *George L. Pickard and William J. Emery. Pergamon Press, New York. 1982. 249 p. £ 17.50, $35 (U.S.) (hard cover); £ 5.95, $11.95 (U.S.) (flexicover).* New features include the elementary treatments of the geostrophic method for current estimations and the process of upwelling, and a brief review of the recent revision of the definition of 'salinity,' in the Practical Salinity Scale 1978. Aimed at senior and graduate students in oceanography, fisheries, and marine biology. [R451]

Aquaculture: the legal framework. *Bruce H. Wildsmith. Edmond-Montgomery Limited, Toronto, Canada. 1982. 313 p. $45.* The objective of this book is to discuss, both as an exposition of present law and in predictive/policy contexts, the full legal framework governing the conduct of aquaculture in Canada. The author has also included information concerning non-Canadian legislation. [R452]

Rainbowfishes of Australia and Papua New Guinea. *Gerald R. Allen and Norbert J. Cross. T. F. H. Publications, Inc., Neptune, New Jersey. 1982. 160 p. with over a hundred full-color photos, plus charts and maps. $16.95 (U.S.).* All eight genera of rainbowfishes are fully discussed including how to keep them in an aquarium and their zoogeography, evolution, and classification. The large ($8\frac{1}{2}'' \times 11''$) format of this hard-covered book allows presentation of many species in larger-than-life colored photos. [R453]

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[1]Persons interested in reviewing books in this list should contact the Book Review Editor, Canadian Journal of Fisheries and Aquatic Sciences, Scientific Information and Publications Branch, Department of Fisheries and Oceans, Ottawa, Canada K1A 0E6.

[1]Les personnes intéressées à examiner les livres énumérés dans cette liste sont priées de communiquer avec le Rédacteur des comptes rendus, Journal canadien des sciences halieutiques et aquatiques, Direction de l'information et des publications scientifiques, Ottawa, Canada K1A 0E6.

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Low pH and the absence of fish species in naturally acidic Wisconsin lakes: inferences for cultural acidification **F. J. Rahel and J. J. Magnuson**

Error bounds for estimates of fish larvae and fish eggs entrained **C. P. Madenjian and D. J. Jude**

Evaluation of the phosphorus–chlorophyll relationship for lakes off the Precambrian Shield in western Canada **E. E. Prepas and D. O. Trew**

Zooplankton diversity and biomass in recently acidified lakes **J. L. Confer, T. Kaaret, and G. E. Likens**

Production modeling using mortality estimates **J. Csirke and J. F. Caddy**

Survival, respiration, and food assimilation of *Daphnia magna* exposed to petroleum- and coal-derived oils at three temperatures **S. O. Ullrich Jr., and R. E. Millemann**

Manipulation of the seasonal reproductive cycle of the landlocked Atlantic salmon (*Salmo salar*) by LHRH analogues administered at various stages of gonadal development **L. W. Crim, D. M. Evans, and B. H. Vickery**

Behavior of crayfish in water currents: hydrodynamics of eight species with reference to their distribution patterns in southern Ontario **S. H. Maude and D. D. Williams**

Le panache du Saguenay **J. Lebel, E. Pelletier, M. Bergeron, N. Belzile et G. Marquis**

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Relationship between body-lead concentration of *Mysis relicta* and sediment-lead concentration in Kootenay Lake, B.C. **R. D. Evans and D. C. Lasenby**

Effect of increased dietary fiber on the growth of rainbow trout (*Salmo gairdneri*) **J. W. Hilton, J. L. Atkinson, and S. J. Slinger**

Total dissolved solids as a predictor of lake biomass and productivity **E. E. Prepas**

Mercury speciation in water **S. Ramamoorthy, T. C. Cheng, and D. J. Kushner**

Use of NaCl as a reference toxicant for goldfish, *Carassius auratus* **R. W. Threader and A. H. Houston**

Consideration of the stable age distribution assumption in "analytical" yield models **M. Sinclair, R. O'Boyle, and T. D. Iles**

Potential sulfonamide therapy of enteric redmouth disease **G. L. Bullock, G. Maestrone, C. Starliper, and B. Schill**

Characteristics and identification of *Pasteurella* and *Vibrio* species pathogenic to fishes using API-20E (Analytab Products) multitube test strips **M. L. Kent** 1725–1729
Book reviews/Comptes rendus: Functional adaptations of marine organisms (F. J. Vernberg and W. B. Vernberg [ed.]) **M. Telford** 1730

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

CJFSDX39(12) 1553–1732 (1982)
ISSN 0706-652X

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Cyclopoid predation on Lake Michigan fish larvae **J. H. Hartig, D. J. Jude, and M. S. Evans** 1563–1568

Predatory behavior and prey selectivity of the pelagic water mite *Piona constricta* **H. P. Riessen** 1569–1579

Susceptibility of trouts of different species and origins to various isolates of infectious pancreatic necrosis virus **A. Silim, M. A. S. Y. Elazhary, and A. Lagacé** 1580–1584

Ingestion-limited growth of aquatic animals: the case for Blackman kinetics **R. E. Condrey** 1585–1595

Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River system **M. Roch, J. A. McCarter, A. T. Matheson, M. J. R. Clark, and R. W. Olafson** 1596–1601

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Meristic variation in beaked redfishes, *Sebastes mentella* and *S. fasciatus*, in the Northwest Atlantic **I-H. Ni** 1664–1685

Reproductive strategies in flatfish: a first synthesis **D. A. Roff** 1686–1698

Characteristics of sediment and organic carbon export from pristine boreal forest watersheds **R. J. Naiman** 1699–1718

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Use of box and line plots to assess fish ventilatory behavior in biological monitoring systems **W. J. Sydor, W. R. Miller III, J. Cairns Jr., and D. Gruber** 1719–1722

Ichthyophthiriasis: immersion immunization of rainbow trout (*Salmo gairdneri*) using *Tetrahymena thermophila* as a protective immunogen **K. Wolf and M. E. Markiw** 1722–1725

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